β^2- and β^3-Peptides with Proteinaceous Side Chains: Synthesis and Solution Structures of Constitutional Isomers, a Novel Helical Secondary Structure and the Influence of Solvation and Hydrophobic Interactions on Folding[1])

by **Dieter Seebach***, **Stefan Abele**[2]), **Karl Gademann**[2]), **Gilles Guichard**[3]), **Tobias Hintermann**[2]), **Bernhard Jaun**, **Jennifer L. Matthews**[4]), and **Jürg V. Schreiber**[5])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

and **Lukas Oberer**, **Ulrich Hommel**, and **Hans Widmer**

Novartis Pharma AG, Research Core Technology Area, CH-4002 Basel

Enantiomerically pure β-amino-acid derivatives with the side chains of Ala, Val, and Leu in the 2- or 3-position (β^2- and β^3-amino acids, resp.), as well as with substituents in both the 2- and 3-positions ($\beta^{2,3}$-amino acids, of *like*-configuration) have been prepared (compounds **8**–**17**) and incorporated (by stepwise synthesis and fragment coupling, intermediates **24**–**34**) into β-hexa-, β-hepta-, and β-dodecapeptides (**1**–**17**). The new and some of the previously prepared β-peptides (**35**–**39**) showed NH/ND exchange rates (in MeOH at room temperature) with $\tau_{1/2}$ values of up to 60 days, unrivalled by short chain α-peptides. All β-peptides **1**–**7** were designed to be able to attain the previously described 3_1-helical structure (*Figs. 1* and *2*). CD Measurements (*Fig. 4*), indicating a new secondary structure of certain β-peptides constructed of β^2- and β^3-amino acids, were confirmed by detailed NMR solution-structure analyses: a β^2-heptapeptide (**2c**) and a $\beta^{2,3}$-hexapeptide (**7c**) have the 3_1-helical structure (*Figs. 6* and *7*), while to a β^2/β^3-hexapeptide (**4**) with alternating substitution pattern H-(β^2-Xaa-β^3-Xaa)$_3$-OH a novel, unusual helical structure (in (D_5)pyridine, *Fig. 8*; and in CD_3OH, *Figs. 9* and *10*) was assigned, with a central ten-membered and two terminal twelve-membered H-bonded rings, and with C=O and N–H bonds pointing alternatively up and down along the axis of the helix (*Fig. 11*). Thus, for the first time, two types of β-peptide turns have been identified in solution. Hydrophobic interactions *of* and hindrance to solvent accessibility *by* the aliphatic side chains are discussed as possible factors influencing the relative stability of the two types of helices.

1. Introduction. – We are used to consider the formation of folds, turns, helices, sheets, and more complex, distinct secondary structures as a domain of the molecules of life. It was, therefore, not really astonishing that science journalists, and others, chose titels such as '*β-Peptides – improving on nature?*'[6]) in accounts on our surprising discovery that short-chain peptide analogs, the β-peptides, consisting of β- rather than α-amino acids form much more stable helices in solution than their natural counterparts, the α-peptides, do [2][4–6]. So far, we have fully described the synthesis and structure of β^3-peptides **A**

[1]) Partially mentioned in preliminary communications [1][2] and in a review article [3].

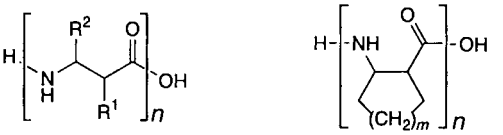
[2]) Part of the projected Ph. D. theses of *S.A.*, *K.G.*, and *T.H.*, ETH-Zürich.

[3]) Grant from the *Association pour la Recherche sur le Cancer* (*ARC*), France. New address: UPR 9021 CNRS, Institut de Biologie Moléculaire et Cellulaire, F-67084 Strasbourg.

[4]) New address: Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ, Scotland.

[5]) Part of the Master Thesis (Diplomarbeit) of *J.S.*, ETH-Zürich, 1997.

[6]) For a short review article on β-peptides, see [3].



A n= 2-12 **B** m= 1,2; n= 4-8

(R^1 = H) [4][5][7], and of cyclic derivatives thereof [4][7][8]. *Gellman* and coworkers have reported, in preliminary form, about β-peptides **B** consisting of 2-aminocycloalkanecarboxylic acids [9]. Disregarding a structure in which the β-amino-acid residues cannot attain a fully staggered conformation of the C(2)–C(3) bond [9b], a 3_1 helix, a

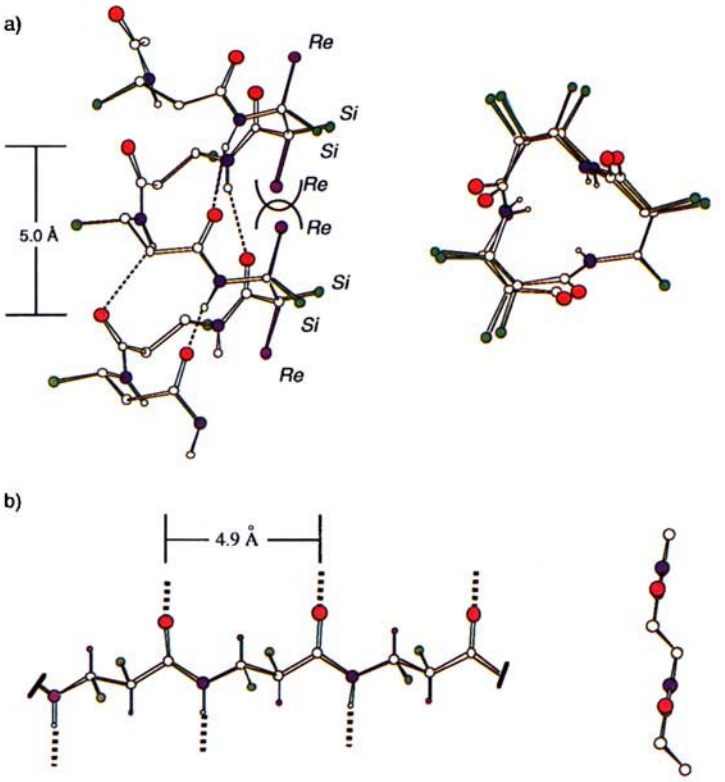


Fig. 1. *Conformations and secondary structures of β-peptides. a)* Left-handed or (*M*)-3_1-helix of a β-peptide in side view and in the top view [4][5][9a]. For steric reasons, non-H-atoms are allowed only in *lateral* positions (green), while the *axial* positions must be occupied by the H-atoms (violet), for the helix to be stable. In a left-handed helix, the β-amino-acid residues must all have a configuration such that their side chains R in 2- or/and 3-position reside in the *Si*-half-space of the corresponding stereogenic center (green, R_{Si}). For a right-handed or (*P*)-helix of this type, the H-atoms must be H_{Si}, the side-chains R_{Re}. *b)* Extended conformation of a β-peptide, side view and top view [4][11]. For H-bonding in a parallel or antiparallel arrangement (*cf.* pleated sheets of α-peptides), the R groups (green) may occupy the positions approximately perpendicular to the amide planes, while non-H-atoms are 'forbidden' in the violet positions ($A^{1,3}$ or 1,3-diaxial strain and disruption of H-bonding would be caused).

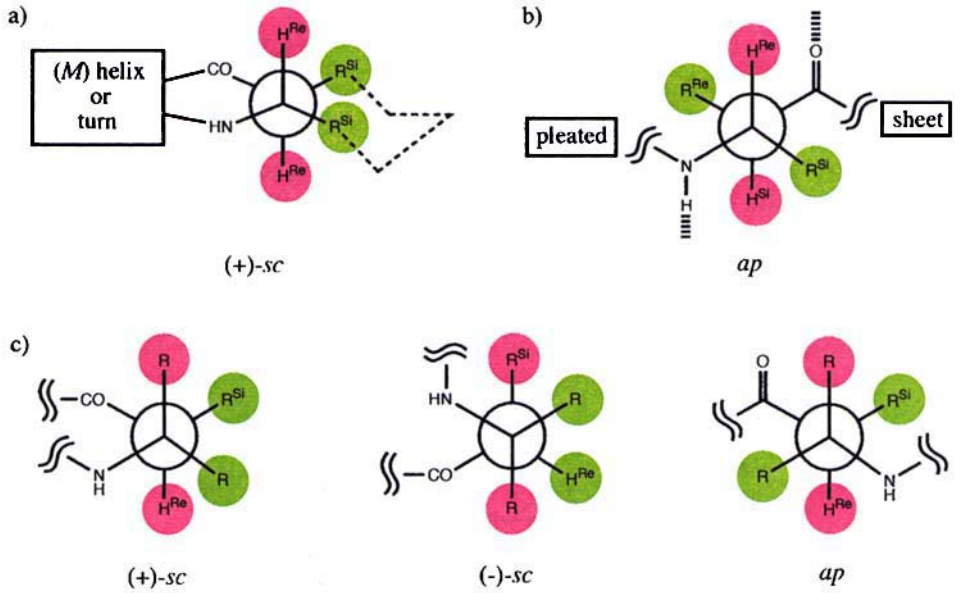


Fig. 2. *The* gauche- *and* anti-*conformations around the C(2)–C(3) bond of β-amino-acid residues.* The positions marked violet must be occupied by H-atoms in the structures as defined in *Fig. 1, a* and *b*. *a*) The (+)-*synclinal*-conformation in the (*M*)-3_1-helix (*cf. Fig. 1, a*) and in turns [4][7]. *b*) The *antiperiplanar* conformation present in a fully extended form of a β-peptide (*cf. Fig. 1, b*). *c*) The three conformers of a geminally 3,3-disubstituted β-amino-acid residue fit neither in a (*M*)- or (*P*)-3_1-helix (one or two R groups *axial*, *i.e.*, parallel to the helix axis, *cf. Fig. 1, a*), nor in an extended form (1,5-repulsion of non-H-atoms or *Newman* strain [12a] between O and R) of a β-peptide. For a conformational analysis, not considering 1,5-repulsion, of a β-amino-acid derivative, see [12b].

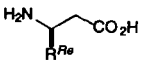
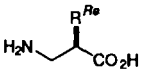
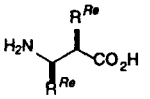
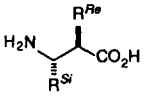
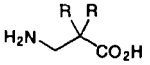
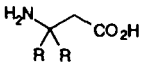
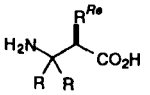
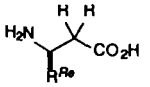
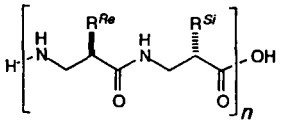
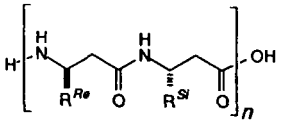
turn[7]), and a parallel sheet-like structure of acyclic β-peptides with 14-membered H-bonded intercatenate rings have been identified in solution (NMR and CD spectroscopy) and in the solid state (X-ray analysis; see *Figs. 1* and *2*)[8]). From this information, the fitting of certain β-amino acids **C**–**K**, and of configurational sequences thereof (**L** and **M**), in the secondary structures can be predicted (*Table 1*). It is the purpose of this paper to describe synthesis and structural investigations of the β-hexa-, β-hepta-, and β-dodecapeptides **1**–**7** with the proteinaceous side chains of Val, Ala, and Leu, with β^2-amino-acid residues (**1** and **2**), with 'mixed' sequences of β^2- and β^3-amino-acid moieties (**3**–**6**), and with disubstituted $\beta^{2,3}$-amino acids (**7**)[9]). *All* β-peptides **1**–**7** were

[7]) The turns, which were identified in crystal structures, of β-di- [7] and β-tripeptides [4] do not involve an intramolecular H-bond (as α-peptide turns do), but can be considered as sections of the 3_1 helix. *Cf.* also the solid-state structures of cyclo-β-tetrapeptides [8].

[8]) After the present work was completed, the structure of a tetrameric antiparallel β-sheet containing two $\beta^{2,3}$-amino acids was published [10].

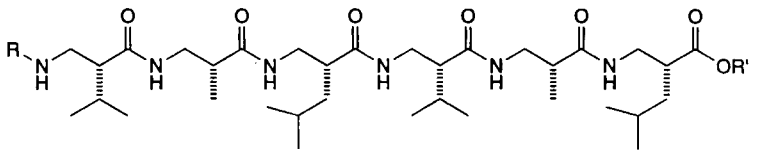
[9]) We use the previously proposed nomenclature H-β^2-HXaa-OH (see **D** in *Table 1*) and H-β^3-HXaa-OH (see **C** in *Table 1*) for the β-amino acids bearing the side-chain in 2- or 3-position, respectively [2][4][5]. For those β-amino acids carrying side chains in 2- and 3-position ($\beta^{2,3}$), the analogy with the natural amino acids is more difficult to indicate (see **E** and **F** in *Table 1* [5], and *Exper. Part*).

Table 1. *Fitting of Various β-Amino Acids* **C**–**K** *and β-Dipeptide Moieties* **L** *and* **M** *in the* 3_1*-Helical or Extended H-Bonded Secondary Structures Shown in* Fig. 1. The configurations are specified by the topicities (*Re*/*Si*) of the side-chains R, because the (*R*/*S*)-specification reverses with the nature of the side-chain in homochiral series (**C** with R = Me, Bn, Me_2CHCH_2 have (*R*)- **C** with R = Me_2CH, $HOCH_2$have (*S*)-configuration!). The same problem may arise with the disubstituted β-amino acids **E** and **F** when using the *l*(*like*)/*u*(*unlike*) specifications. With the aldol convention, **E** and *ent*-**E** would be *anti*, **F** and *ent*-**F** *syn*. The ✓ and – signs indicate that the corresponding β-amino-acid residues fit or do not fit into the 3_1-helical or sheet-like structures shown in *Fig. 1*; the question mark means that no secondary structure has been identified for β-peptides containing the building blocks **G**–**M**. **L** and **M** are just two examples of 'misfitting' sequences which can be imagined.

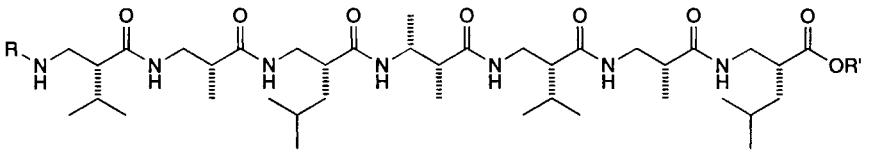
β-Peptide built from β-amino acid **C**–**K** or β-dipeptide fragment **L**, **M**		Fit in Secondary Structure			
		3_1-Helix		Parallel or antiparallel sheet	Neither 3_1-helix nor sheet
		(*P*)	(*M*)		
	**C**	✓	–	✓	
	ent-**C**	–	✓	✓	
	**D**	✓	–	✓	
	ent-**D**	–	✓	✓	
	**E**	✓	–	–	
	ent-**E**	–	✓	–	
	**F**	–	–	✓	
	ent-**F**	–	–	✓	
	**G**	–	–	–	?
	**H**	–	–	–	?
	**I**	–	–	–	?
	ent-**I**	–	–	–	?
	**K**	–	–	–	?
	ent-**K**	–	–	–	?
	**L**	–	–	–	?
	**M**	–	–	–	?

designed so that they *can* form an (*M*)- (**3**–**7**) or (*P*)- (**1**, **2**) 3_1-helical secondary structure (with *all* side-chains in the 'allowed', lateral positions), and it is, perhaps, the most important result described herein, that some of them *do not*!

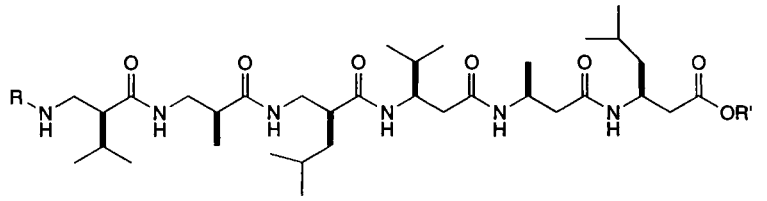
1



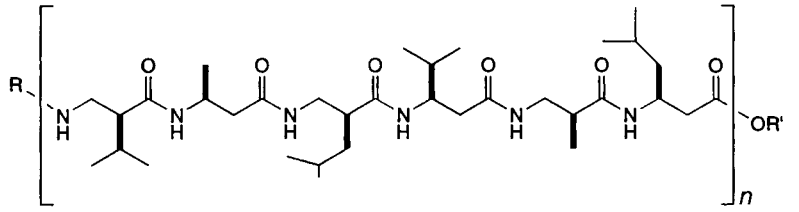
2



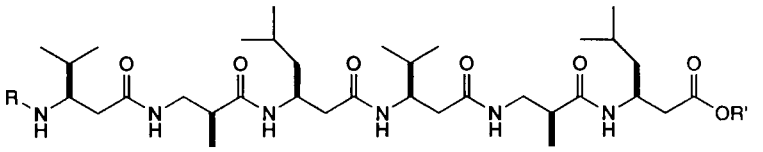
3



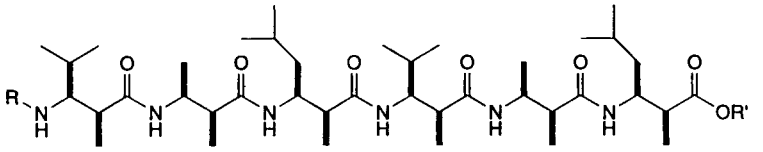
4 $n = 1$
5 $n = 2$



6

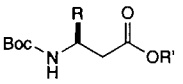


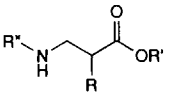
7



a R = Boc, R' = Bn
b R = H, R' = Bn, as trifluoroacetate
c R = R' = H, as trifluoroacetate
d R = R' = H, as hydrochloride

2. Preparation of the β^2- and $\beta^{2,3}$-Amino-Acid Building Blocks. – For constructing the seven β-peptides, the β^2-, β^3-, and $\beta^{2,3}$-amino-acid derivatives **8**–**16** with the side-chains of alanine (**a**), valine (**b**), and leucine (**c**) had to be prepared in enantiomerically pure form. The Boc-protected β^3-amino acids, **8a**–**8c**, and their benzyl esters were obtained from the corresponding α-amino acids by *Arndt-Eistert* homologation, as described in [13–16], and so were the corresponding methyl esters **10a**–**10c**, which served as starting materials for α-methylations (*Scheme 1*). Of the methods now available for the EPC synthesis of 2-substituted 3-amino-carboxylic acids [17], we chose the α-methylation of Boc-β^3-HAla-OMe, Boc-β^3-HVal-OMe, and Boc-β^3-HLeu-OMe [4] through doubly lithiated derivatives **N**, a procedure which we had developed many years ago [14][18][19]. With 3-amino-*N*-benzoylbutanoates and in the presence of LiCl [20], the reaction is highly selective with relative topicity *lk*. The Boc derivatives, which undergo methylation (→ **14**/*epi*-**14**) with poor selectivity (3:1–1:3), were used in the present

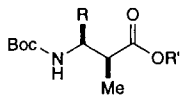




(*R*)- or (*S*)-configuration

8 R' = H
9 R' = Bn
10 R' = Me

11 R' = R" = H
12 R' = Bn, R" = H (as tosylate salt)
13 R' = H, R" = Boc



14 R' = Me
15 R' = Bn
16 R' = H

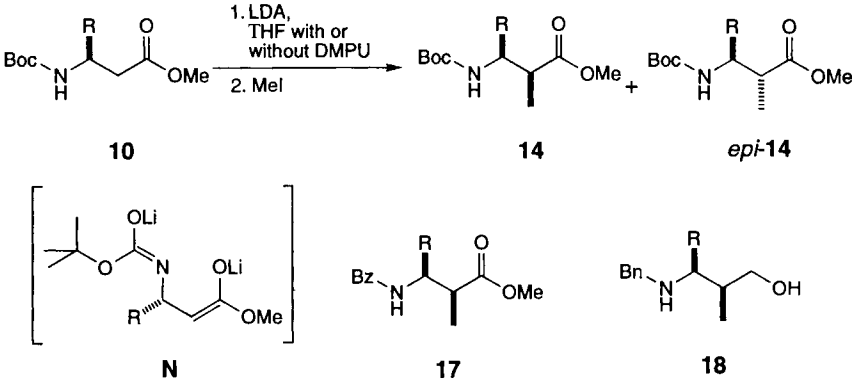
a R = Me; **b** R = Me_2CH; **c** R = Me_2CHCH_2; **d** R = $PhCH_2$

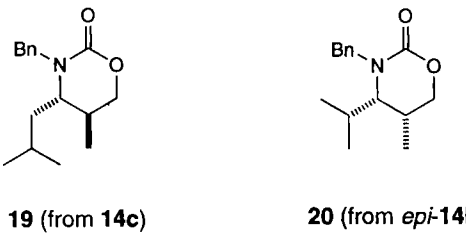
investigation for the simple reason that we needed to have both epimers[10]) (*Scheme 1*). Epimer **14** was formed preferentially in THF solvent, 2-*epi*-**14** in THF/DMPU mixtures. The epimers **14a**/*epi*-**14a** [5] can be separated by HPLC, while the new compounds **14b**, *epi*-**14b**, and **14c**, *epi*-**14c** were isolated in pure form by flash chromatography. The configuration of the epimer **14a** was known [19], and that of **14b** and **14c** was assigned by conversion to heterocyclic carbamates such as **19** and **20** (*Scheme 1*) of which we have determined the crystal structures (*Fig. 3*); the *trans*- and *cis*-substituted heterocycles show characteristic NOEs in their ^{1}H-NMR spectra which could be used for configurational assignments of other derivatives (see *Exper. Part*). The Boc-protected benzyl esters **15**, required for the β-peptide synthesis[11]), were prepared from the methyl esters **14**

[10]) β-Amino-acid derivatives with the configuration of 2-*epi*-**14** are of the type **F** in *Table 1*, and thus should not fit into the 3_1-helical structure, but, rather, into the extended conformation of β-peptides (*Figs. 1* and *2*). Indeed, a tripeptide prepared from 2-*epi*-**14a**, **b**, and **c** was insoluble 'like a rock' (except in protic solvent), indicating strong intermolecular H-bonding (sheets as described in [4][10][21]).

[11]) Alkaline saponification of the methyl esters **14** and of β-peptides containing such α-substituted β-amino acids leads to partial epimerizations.

Scheme 1. *Preparation and Configurational Assignment of the α-Methyl-Substituted β-Amino Acid Derivatives* **14**. The methylation reactions were carried out in *ca.* 30-mmol batches (yields of epimer mixtures up to 90%). The configuration of the enolate and imino-carboxylate C=C and C=N bonds in **N** is unknown and is drawn arbitrarily. The 1,3-oxazinan-2-ones **19** and **20** were prepared from **14c** and *epi*-**14b**, respectively, in the following way: *i*) Boc deprotection, *ii*) N-benzoylation (→ **17c**, *epi*-**17b**), *iii*) $LiAlH_4$ reduction to the corresponding β-amino-alcohols (→ **18c**, *epi*-**18b**), *iv*) cyclization with $(Cl_3CO)_2CO$ (see *Exper. Part*).





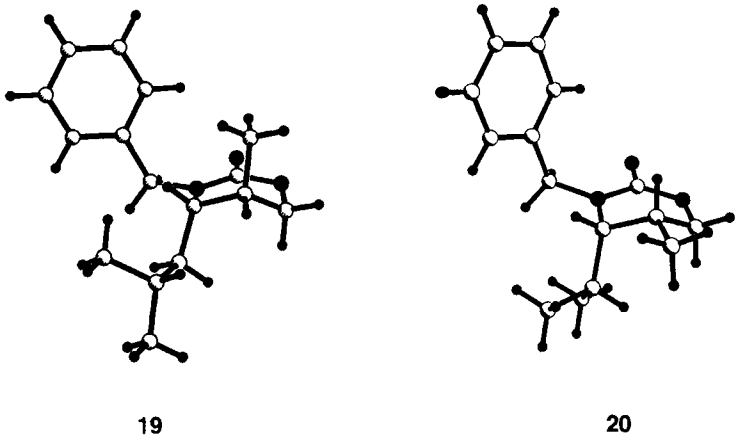
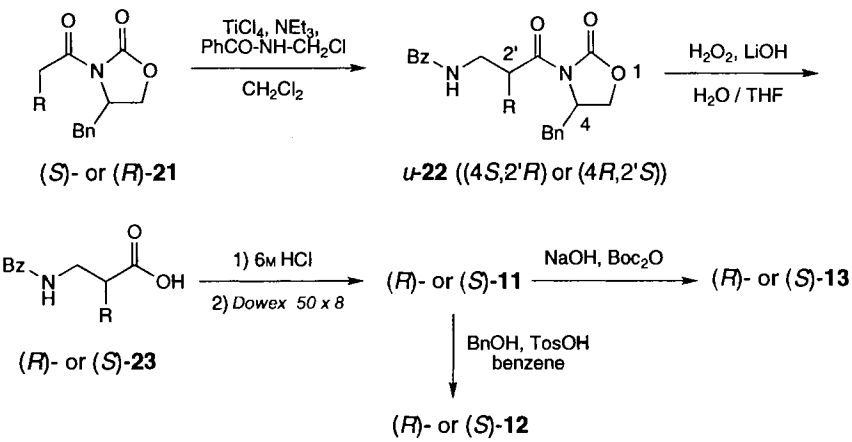


Fig. 3. *PLUTO Plots of the X-ray crystal structures of* **19** *and* **20**. The structures were determined by *V. Gramlich* (crystallographic laboratory course of ETH) and *P. Seiler* (X-ray service, Laboratorium für Organische Chemie, ETH).

by titanate-mediated transesterification [22][12]), and the benzyl esters were cleaved hydrogenolytically, as needed (→ **16a–c**).

The β^2-amino acids **11a–c** are not accessible from α-amino acids. Of the methods available [15], we decided to use *Evans'* enolate chemistry as outlined in *Scheme 2*: in a paper on alkylations of *N*-acyl-oxazolidinones through $TiCl_4$-enolates, one of the electrophiles employed was (benzoylamino)methyl chloride which led to a β^2-amino-acid derivative of type **22** [24]. Thus, we used the (*S*)- and (*R*)-3-propanoyl-, 3-isopentanoyl-, 3-(4-methylpentanoyl)-, and 3-(3-phenylpropanoyl)-4-benzyl-1,3-oxazolidin-2-ones **21** for the amidomethylation and obtained the products *u*-**22** with diastereoselectivities from 93:7 to 99:1. Hydrolytic removal of the auxiliary group with $LiOH/H_2O_2$ [25] and debenzoylation in refluxing acid, followed by ion-exchange chromatography [19], led to the free amino acids **11** which were converted to benzyl esters **12** and to Boc derivatives **13** for the peptide synthesis.

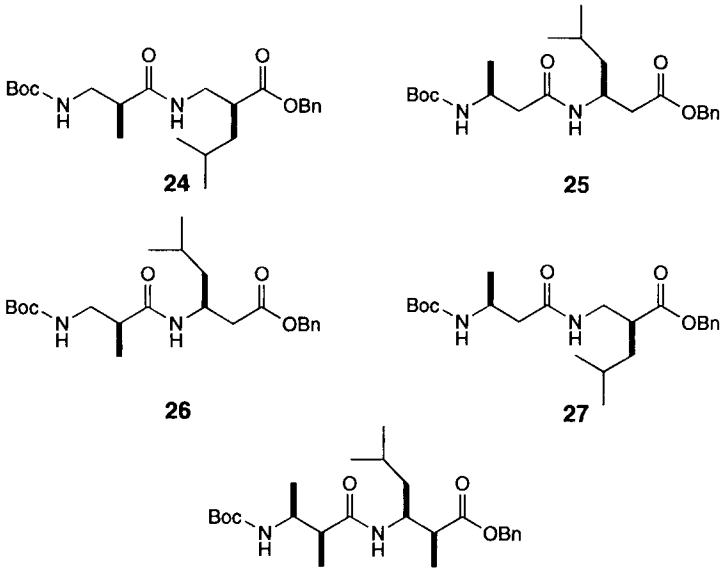
Scheme 2. *Preparation of (R)- or (S)-β²-Amino-Acid Derivatives by Aminomethylations of Acyl-oxazolidinones*



a R = Me; **b** R = Me_2CH; **c** R = Me_2CHCH_2; **d** R = $PhCH_2$

3. Synthesis of β-Peptides. – The β-peptides were synthesized in solution by conventional peptide-coupling methods with EDC/HOBt [4][5]. To reduce epimerization during the synthesis of β^2- and $\beta^{2,3}$-peptides, Et_3N was replaced by the weaker base NMM. Coupling of the tosylate salt of H-β^2-HLeu-OBn (**12c**) with Boc-β^2-HAla-OH (**13c**) or Boc-β^3-HAla-OH (**8a**) led to the β-dipeptides **24** and **27**. Boc-β^3-HLeu-OBn (**9c**) was deprotected with CF_3COOH (TFA) and coupled with the Boc-protected β^3- and β^2-amino acids **8a** and **13a** to yield the β-dipeptides **25** and **26**, respectively. Similarly, Boc-deprotection of the $\beta^{2,3}$-amino acid benzyl ester **15c** and coupling with the Boc-protected $\beta^{2,3}$-amino acid **16a** gave the $\beta^{2,3}$-dipeptide **28**.

[12]) Although this method is generally considered as safe concerning carbamate protection, we saw traces of Z-protected benzyl esters in the mass spectra of crude products. For titanate-mediated protecting group manipulations, see [23].



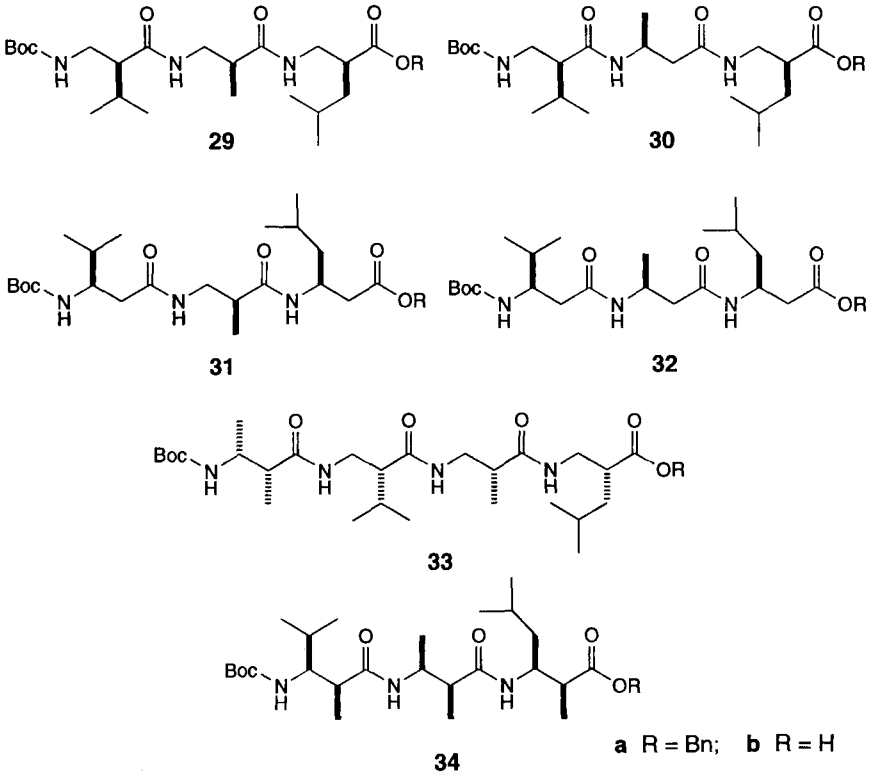
24 **25** **26** **27** **28**

The β-dipeptides **24**–**28** were *N*-deprotected with TFA or HCl/dioxane, and coupled with the Boc-β-amino acids **8b**, **13b**, or **16b** to give the β-tripeptides **29a**–**32a**, and **34a**. The yields for the coupling steps to the β-di- and β-tripeptides were good-to-excellent (75–92%). In the case of the $\beta^{2,3}$-peptides **28** and **34**, the yield could be substantially increased (81 and 85%) by employing the free amino ester instead of the CF_3COOH salt in the coupling step.

β-Tripeptides were deprotected on the C-terminus by hydrogenolysis (H_2, Pd/C) and then coupled with the corresponding *N*-deprotected tripeptides to give the β-hexapeptides **1a**, **3a**, **4a**, **6a**, and **7a**. For the preparation of the β^2-heptapeptide **2**, the tripeptide derivative *ent*-**29a** was *N*-deprotected and coupled with the Boc-$\beta^{2,3}$-amino acid *ent*-**16a** to the β-tetrapeptide **33a**. *N*-Deprotection of **33a** and fragment coupling with *ent*-**29b** gave the β^2-heptapeptide **2**. The good solubility of the mixed β-hexapeptide derivative **4a** (*e.g.*, in AcOEt) encouraged us to synthesize the β-dodecapeptide **5a**. Thus, the fully protected β-hexapeptide **4a** was debenzylated (H_2, Pd/C) and coupled with the Boc-deprotected derivative of β-hexapeptide **4a** in 74% yield (crude product).

In contrast to the fully protected β^3-hexa- and β^3-heptapeptides [4][5], the β^2-, the mixed β-hexa- and β-heptapeptides, and the $\beta^{2,3}$-hexapeptide are well soluble in organic solvents ($CHCl_3$, MeOH) and can be purified by flash chromatography (FC). The yields for the fragment coupling reactions were in the range of 37–69%. In the case of coupling β^2- and, especially, $\beta^{2,3}$-amino acids, epimerization was observed, but separation of the resulting epimers was readily achieved by recrystallization or FC[13]). We have also

[13]) NMR Analysis of the major impurity formed with the $\beta^{2,3}$-hexapeptide **7a** revealed that *ca.* 20% epimerization had taken place.



observed some epimerizaton in the solid-phase synthesis of β^2-peptides [26]. All peptides **1**–**7** were fully characterized by mass, NMR, and IR spectroscopy, and correct elemental analyses were obtained for the hexapeptides **4a** and **7a**.

The free β-peptides **1c**, **2c**, **3c**, **4c**, **5c**, and **7c** were isolated as TFA salts[14]) by hydrogenolysis (H_2/Pd) and Boc deprotection of the corresponding precursors. They were further purified by RP-HPLC (see *Exper. Part*) to produce samples which were used for conformational analysis by CD and NMR spectroscopy.

4. Structural Analysis. – 4.1. ***Circular Dichroism Spectroscopy.*** Circular dichroism (CD) is extensively used to obtain information on the secondary structure of peptides and proteins in solution. For α-peptides and proteins, characteristic CD patterns can be assigned to β-sheet and α-helix structures [27]. However, in the world of β-peptides, the correlation between CD patterns and types of secondary structure is yet to be established. The results of efforts made with the goal to predict the CD spectra of poly(β-amino acids) on the basis of theoretical calculations have been published [28]. In our own previous work, we have identified and characterized a 3_1-helical structure of various β-peptides [2][4][5][26] by CD spectroscopy in MeOH solution. Another type of secondary structure, causing a distinct CD pattern of β-peptides (consisting of *trans*-2-aminocyclopentanecarboxylic acid) [10], has been identified as 2.5_1 helix [3].

[14]) With the exception of **4d**, which was isolated as HCl salt.

Since all peptides **1**–**7** synthesized and described herein comply with the configurational requirements for the formation of a 3_1 helix (*i.e.*, all side chains could occupy lateral position on a 3_1-helical conformation; see *Table 1*), we expected to see the typical CD pattern (extrema of opposite sign near 215 and near 200 nm) in all cases. For β^3- and $\beta^{2,3}$-peptides (**3**–**7**), containing β-amino acids derived from L- or (*S*)-α-amino acids or composed of (*S*)-β^2-amino acids, the longer-wavelength *Cotton* effect ought to be negative. Indeed, the deprotected β-peptides **1c**, **2c**, **3c**, **5c**, and **7c** show the CD curves[15]) indicative of 3_1-helical conformations (*Fig. 4*). While the β^2-hexapeptide **1c** (from (*R*)-β^2-amino acids) shows a rather weak positive *Cotton* effect, the β^2-heptapeptide **2c**, containing an additional, central (2*R*,3*R*)-$\beta^{2,3}$-amino acid has a much stronger maximum at 215 nm (molar ellipticity $2.5 \cdot 10^4$ *vs.* $1.1 \cdot 10^5$). The record molar ellipticity for a β-hexapeptide was measured with the all-*like*-$\beta^{2,3}$-hexapeptide **7c** ($1.5 \cdot 10^5$ at 198 nm as compared to $5.9 \cdot 10^4$ for the β^3-analogue without α-methyl substituents [4], *Fig. 4,a*).

Amazingly, the fully protected mixed β-peptides **3a**–**6a** did not show the familiar 215/200-nm CD pattern in MeOH solution, but a new type of CD spectrum with an intense single peak at *ca.* 205 nm (*Fig. 4,b* and *c*). Thus, these *mixed* β-peptides must have a secondary structure different from that of the positional isomers in which *all* side chains are in the 2- or in the 3-position of the β-amino-acid residues, in spite of the fact that their structures would fit into the (*M*)-3_1-helix with *all*-lateral substituents. Furthermore, the mixed β-peptides exhibit a subtle dependence of the CD spectra from the protecting groups: upon deprotection of **3a**, **4a**, and **5a** to **3c**, **4c**, and **5c**, respectively, the familiar pattern with negative longer, and positive shorter wavelength *Cotton* effect was restored for **3c** and **5c**, but not for **4c** (*Fig. 4,b* and *c*). Thus, the enormous 205-nm positive *Cotton* effect ($6 \cdot 10^5$) of the *N*-Boc- and benzyl-ester-protected dodecapeptide **5a** collapses to a meager 215 ($-2.7 \cdot 10^4$)/200 ($+ 4.9 \cdot 10^4$) CD pattern upon deprotection (*Fig. 4,c*). Only the hexapeptide **4** shows the new type of CD spectrum, irrespective of whether its termini are protected or not (*Fig. 4,c*). Thus, we chose this β-peptide as one of the candidates for elaborate NMR analyses, described in *Sect. 4.2.2.*

As reported previously [4][5], the CD spectra of β-peptides were found to be concentration-independent within the range studied. This also holds for the β-peptides **2c** and **3c**. Surprisingly, the most highly substituted β-peptide **7c** gives rise to concentration-dependent CD spectra, indicative of an aggregation phenomenon. We have also studied the temperature dependence of the CD spectrum of one of the β-peptides: the weak *Cotton* effect of the β^2-hexapeptide **1c** increased from $\Theta = + 2.0 \cdot 10^4$ to $+ 2.8 \cdot 10^4$, when going from room temperature to $-20°$[16]).

4.2. ***NMR-Spectroscopic Measurements.*** 4.2.1. *One-Dimensional* ^{1}*H-NMR Spectra and NH/ND Exchange Rates.* The kinetics of amide proton H/D exchange in peptides can give useful informations concerning the solvent accessibility of the amide proton and the dynamics of protein folding [29]. In native proteins, amide protons found in α-helices and

---

[15]) The CD measurements were performed either with lyophylized samples or with samples dried under high vacuum. The TFA content in the samples was not determined.

[16]) We have just started a systematic CD and NMR investigation of β-peptides at different temperatures and concentrations. This and the results of microcalorimetric measurements will provide thermodynamic parameters for the folding (and possible aggregations) of β-peptides and will allow us to compare measured with calculated values [6] (see also [37]).

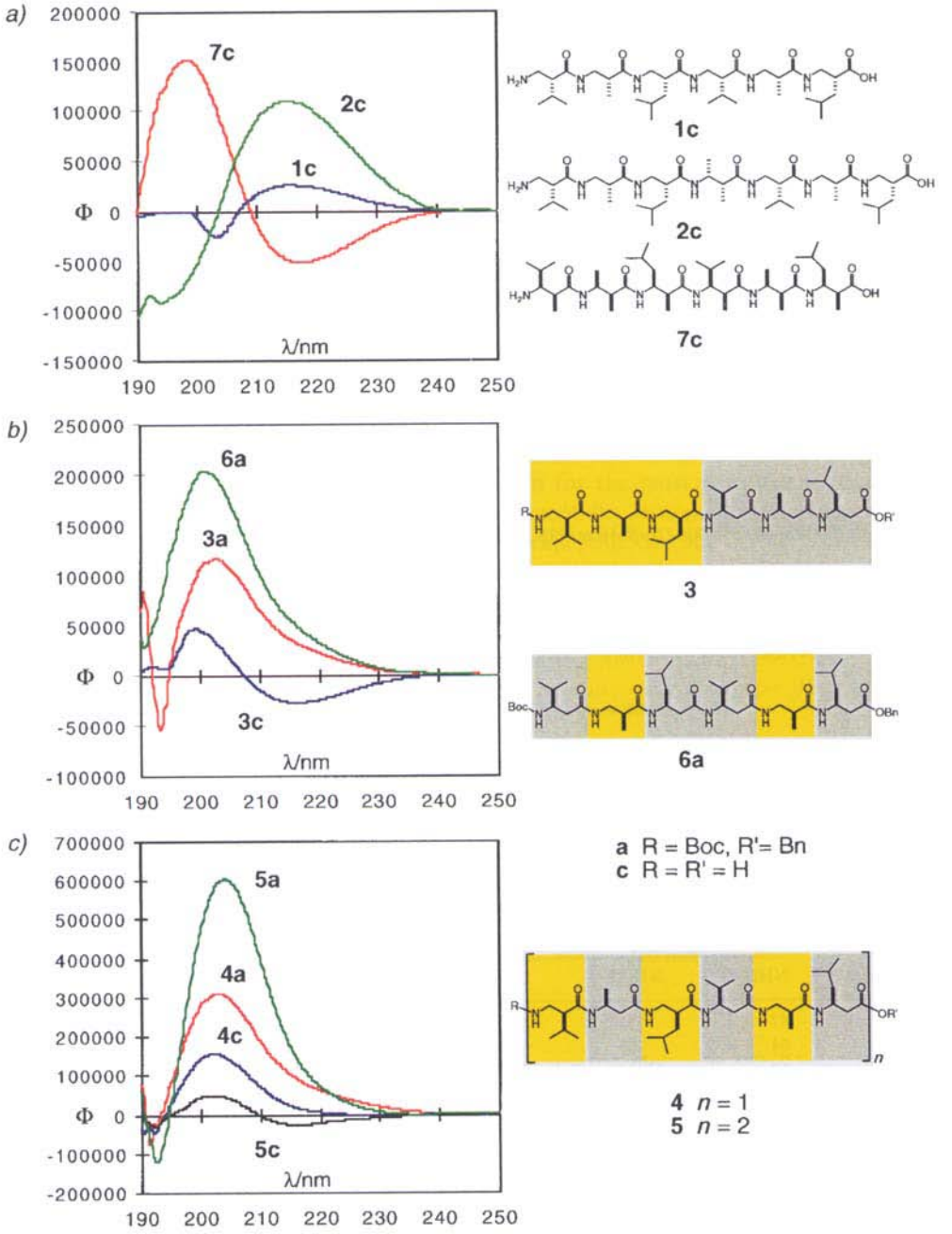


Fig. 4. *CD Spectra of terminally protected and unprotected β-peptides.* a) *CD Curves of unprotected* β^2*-hexapeptide* **1c**, *β-heptapeptide* **2c**, *and all-*like-$\beta^{2,3}$*-hexapeptide* **7c**. b) *CD Spectra of the protected and unprotected mixed β-hexapeptides* **3a** *and* **3c**, *and the protected β-hexapeptide* **6a**. c) *Overlay of the CD spectra of the alternating β-hexa- and β-dodecapeptides* **4a**,**c** *and* **5a**,**c**. All β-peptides were measured as 0.2 mM solutions in MeOH. Molar ellipticity [Φ] in 10 deg · cm^2 · mol^{-1}. The deprotected β-peptides were measured as their TFA salts as obtained after lyophilization or drying under high vacuum [15]).

β-sheets are likely to be strongly protected against H/D exchange. We have determined the half-lives of the exchange of amide protons in CD_3OD for the TFA salts of the β-hexapeptides **3c**, **4c**, **7c**, of the β-heptapeptides **2c**, **35**–**38**, and of the pentadecapeptide **39**[17]). The results are summarized in *Tables 2* and *3*. In the case of peptides **2c**, **4c**, **7c**, and **38**, the amide proton resonances were assigned to the specific amino acids by 2D-NMR techniques (*Sect. 4.2.2*). For the peptides **3c** and **35**–**37**, the amide proton signals were not assigned.

H-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-(*S*)-β^3-HPhe-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH

35

H-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLys-(*S*)-β^3-HPhe-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLys-OH

36

H-(*S*)-β^2-HVal-(*S*)-β^2-HAla-(*S*)-β^2-HLeu-(*S*)-β^2-HPhe-(*S*)-β^2-HVal-(*S*)-β^2-HAla-(*S*)-β^2-HLeu-OH

37

H-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH

38

H-(*R*)-β^3-HLeu-(*S*)-β^3-HAla-(*S*)-β^3-HVal-(*S*)-β^3-HPhe-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-(*S*)-β^3-HPhe-(*R*)-β^3-HLeu-(*S*)-β^3-HAla-(*S*)-β^3-HVal-(*S*)-β^3-HPhe-(*R*)-β^3-HVal-(*S*)-β^3-HAla-(*S*)-β^3-HLeu-OH

39

While the H/D exchange of the N-terminal amine protons was too fast to be detected by simple ^{1}H-NMR measurements, the exchange rate constants for most of the amide protons could be determined[18]).

Table 2. *Half-ife Values* $\tau_{1/2}$ *of Assigned NH Protons in Peptides* **2c**, **4c**, **7c**, *and* **38** *at 25° in* CD_3OD

Peptides	$\tau_{1/2}$ [min]					
	NH(2)[a])	NH(3)	NH(4)	NH(5)	NH(6)	NH(7)
2c	87	81	258	177	111	106
4c	64	68	105	68	140	–
7c	60	13401 (*ca.* 9 d)	1866	117	112	–
38	5	188	230	262	37	40

[a]) The amide protons are numbered according to their position in the sequence starting from the N-terminus of the peptide.

[17]) Synthesis and structural investigations of the peptides **35**–**37**, **39** [26], and **38** [5] have been already published.

[18]) All NMR measurements were performed in commercially available CD_3OD. The concentrations were in the range of 10–16 mg of peptide in 0.7 ml of CD_3OD. Owing to lyophilization or drying under high vacuum, the amount of TFA should be ≤ 1 equiv. The rate constants were calculated as outlined in the *Exper. Part.*

Table 3. *Half-Life Values* $\tau_{1/2}$ *of Unassigned NH Protons in Peptides* **3c**, **35**–**37** *at 25° in* CD_3OD

Peptide	δ [ppm]	$\tau_{1/2}$ [min]	Peptide	δ [ppm]	$\tau_{1/2}$ [min]
3c	8.46	85	**35**	8.43	106
	8.37	144		8.37	51
	7.82	134		7.74	19
	7.73	125		7.69	80
				7.48	90
36	8.42	60	**37**	8.47	<30
	8.32	51		8.43	90
	7.95	57		8.33	41
	7.78	74		8.05	72
	7.74	111		7.57	115
	7.71	<10			

A comparison of the half-lives ($\tau_{1/2}$) of amide proton exchange for β-peptides containing only β^2- and/or β^3-amino acids shows that the values are in the range of 5–144 min with typical half-lives of 106–144 min for the most perseverant protons. Similar rates of exchange were observed for hexapeptides **3c** and **4c** *vs.* heptapeptides **35**–**37**. However, measurement with the pentadecapeptide **39** (one of the protons requires *ca.* 59 days for half exchange)[19]) shows that increasing the peptide length reduces the rate of exchange drastically, a well-known phenomenon with α-peptides (*Fig. 5*) [30].

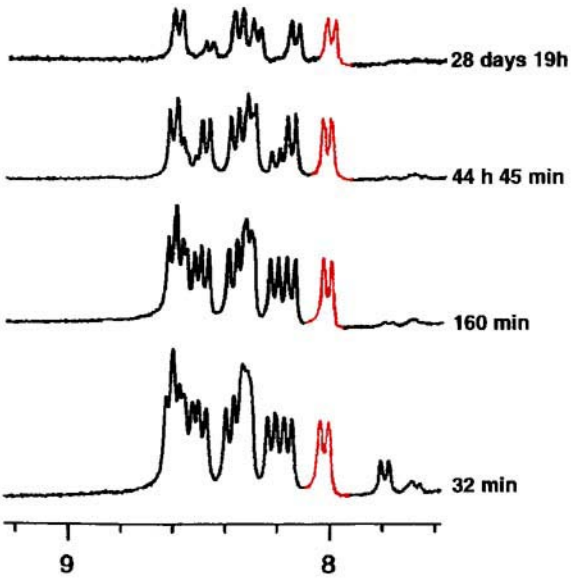


Fig. 5. *Amide proton exchange kinetics of the pentadecapeptide* **39** *at 25° in* CD_3OD. The half-life $\tau_{1/2}$ = 59 days of the red signal (δ = 8.02 ppm) was determined as described in the *Exper. Part.*

[19]) Two amide protons of the hexameric *trans*-2-aminocyclohexanecarboxylic acid required more than two days for complete exchange in CD_3OD [9a].

The 3_1-helical peptides **3c** and **35**–**37** have exchange rates in the same range as the mixed β-peptide **4c**, which has a totally different secondary structure (*Sect. 4.2.2.3*). When the values for the β^2-peptide **35**, the β^3-peptide **36**, and the mixed β^2/β^3-peptides **3c** and **4c** are compared, it appears that neither the type nor the sequence of monosubstituted β-amino acids seems to influence the kinetics of amide proton exchange substantially. The β-heptapeptides **2c** and **38** that contain one $\beta^{2,3}$-amino acid in the middle part of the sequence show maximal half-life values of *ca.* 260 min which correspond to a twofold increase compared to the aforementioned peptides. Still longer half-lives – up to 9 days! – were found for the amide protons in the β-hexapeptide **7c** which is built completely from $\beta^{2,3}$-amino acids.

Several tendencies can be deduced from *Tables 2* and *3*: *1*) In the peptides that adopt 3_1-helical conformations, the amide protons in the middle of the β-peptide sequence have generally longer half-lives than those located near the C- and, especially, near the N-terminus. *2*) The slower exchange kinetics observed for the amide protons at the C-termini compared to those at the N-termini indicate that not the involvement in intramolecular H-bonding but rather the diminished solvent accessibility of the NH protons is responsible for the difference. *3*) The local environment of the amide bond has a strong influence on the exchange kinetics: among the peptides studied by 2D-NMR, the amide group with the slowest H/D exchange rate is surrounded by two side chains[20]). In the $\beta^{2,3}$-hexapeptide **7c**, the combination of this very efficient local shielding effect together with the specific shielding due to the 3_1 helix leads to an extremely slow exchange with a half-life of 9 days for NH(3) (numbering from N- to C-terminus).

4.2.2. *Determination of the Solution Structure of Three New β-Peptides by 2D-NMR Spectroscopy.* In previous full papers [4][5], we have described the NMR solution structures of β^3-hexa- and β^3-heptapeptides (containing only β^3-amino-acid residues). Elaborate NMR analyses have now been performed with three novel β-peptides: the β^2-heptapeptide **2c** with a central $\beta^{2,3}$-amino acid (as TFA salt in CD_3OH), the $\beta^{2,3}$-hexapeptide **7c** with substituents at every backbone C-atom (as TFA salt in CD_3OH), and the β-hexapeptide **4** with alternating β^2- and β^3-amino-acid residues (as **4d** · HCl in (D_5)pyridine and as TFA salt **4c** in CD_3OH).

4.2.2.1. *NMR Structure of the Heptapeptide* **2c** *in* CD_3OH. As can be seen by comparison of the CD spectra of the β^2-hexa- and β^2-heptapeptide **1c** and **2c**, the secondary structure in the latter is much more pronounced (a fact noticed before with the analogous β^3-peptides [4][5]). We, therefore, chose **2c** for a detailed NMR analysis using DQF-COSY, HSQC, HMBC, and ROESY experiments. DQF-COSY and HSQC techniques allowed the assignment of all resonances in the ^1H spectrum as well as of all H-bearing C-atoms in the ^{13}C spectrum. An HMBC experiment was performed in order to assign the sequence through C,H long-range correlations across the peptide bond. The resulting ^1H chemical shifts and the $^1H,^1H$ coupling constants are collected in *Table 4*, the ^{13}C chemical shifts in *Table 5*.

The backbone CH_2 protons in β^2- and β^3-peptides allow to extract valuable and precise information about the backbone torsion angles from ^3J coupling constants. Thus,

---

[20]) Large substituents (Me_2CH, Me_2CHCH_2) are better suited for shielding than the small Me substituent. Solvent accessibility is greater for amide bonds with a neighboring CH_2 group, either on the carboxy or the amino side of the peptide bond.

Table 4. *^{1}H-NMR Chemical Shifts* (CD_3OH) *and Coupling Constants for the Heptapeptide* **2c**. Diastereotopic CH_2 protons and Me groups (on valine and leucine side chains) are labeled with primes, or in italics in the order of decreasing ^{1}H chemical shifts.

Residue	β^2-HVal[1]	β^2-HAla[2]	β^2-HLeu[3]	β-Me-β^2-HAla[4]	β^2-HVal[5]	β^2-HAla[6]	β^2-HLeu[7]
NH, NH_3	7.71 (br.) –	8.58 (J(NH,βax) = 9.2; J(NH,βlat) < 1.5)	8.41 (J(NH,βax) = 8.9; J(NH,βlat) = 1.2)	8.48 (J(NH,βax) = 9.1) –	7.51 (J(NH,βax) = 9.1; J(NH,βlat) = 1.6)	7.77 (J(NH,βax) = 8.5; (J(NH,βlat) < 2)	8.03 (J(NH,βax) = 7.0; J(NH,βlat = 4.5)
H_{ax}–C(β)	3.18 (J(βax,αax) = 9.9) –	3.92 (J(βax,αax) = 11.9; J(β,β) = 12.8)	3.65 (J(βax,αax) = 11.5; J(β,β) = 12.8)	4.13 (J(βax,αax) = 10.2) –	3.86 (J(βax,αax) = 11.9; J(β,β) = 13.0)	2.63 (J(βax,αax) = 11.1; J(β,β) = 13.4)	3.49 (J(βax,αax) = 10.0; J(β,β) = 13.2)
H_{lat}–C(β)	3.18	2.89	2.90	–	2.96	2.82	3.23
CH_3–C(β)	–	–	–	1.16 (J = 6.7)	–	–	–
H_{ax}–C(α)	2.75 (J(αax,β′) = 7.8)	3.14 (J(αax,β′) = 7.0)	2.86	2.59	2.27 (J(αax,β′) = 7.3)	2.63 (J(αax,β′) = 7.1)	2.76
CH_3–C(α)	–	1.08	–	1.13 (J = 7.0)	–	1.11	–
H–C(β′)	1.85 (J = 4.8)	–	–	–	1.76	–	–
H*H*–C(β′)	–	–	1.46	–	–	–	1.50
*H*H–C(β′)	–	–	1.22	–	–	–	1.30
H–C(γ)	–	–	1.56	–	–	–	1.64
CH_3–C(γ)	1.00 (J = 6.8)	–	–	–	0.98 (J = 6.8)	–	–
CH_3'–C(γ)	0.97 (J = 6.7)	–	–	–	0.95 (J = 6.8)	–	–
CH_3–C(δ)	–	–	0.93 (J = 6.9)	–	–	–	0.94 (J = 6.7)
CH_3'–C(δ)	–	–	0.90 (J = 6.6)	–	–	–	0.93 (J = 6.6)

Table 5. *^{13}C-NMR Chemical Shifts* (CD_3OH) *for the Heptapeptide* **2c**. Diastereotopic Me groups (on valine and leucine side chains) are labeled with primes in the order of decreasing chemical shifts.

Residue	β^2-HVal[1]	β^2-HAla[2]	β^2-HLeu[3]	β-Me-β^2-HAla[4]	β^2-HVal[5]	β^2-HAla[6]	β^2-HLeu[7]
C(α)	52.1	41.5	45.1	47.0	53.9	41.4	44.8
C(β)	41.2	43.0	42.4	48.7	40.1	42.7	42.6
$CH_3(\beta')$	–	15.9	–	16.6	–	16.8	–
$CH_2(\beta')$	–	–	41.7	–	–	–	40.9
$CH(\beta')$	30.5	–	–	–	31.1	–	–
$CH_3(\gamma')$	20.5	–	–	18.7	20.9	–	–
$CH_3'(\gamma')$	20.3	–	–	–	20.9	–	–
$CH(\gamma')$	–	–	27.0	–	–	–	27.2
$CH_3(\delta)$	–	–	23.7	–	–	–	22.8
$CH_3'(\delta)$	–	–	23.4	–	–	–	22.3
C=O	173.9	177.6	175.4	176.3	174.7	176.2	178.6

it is evident from the large ^3J(NH, H–C(β)) (7.0–9.2 Hz) and ^3J(H–C(α), H–C(β)) (9.9–11.9 Hz) values that the rotation around the N–C(β) and C(β)–C(α) bonds in **2c** is strongly restricted, and that the protons specified above must be situated in a nearly *antiperiplanar* arrangement[21]). Furthermore, the slow H/D exchange of the NH protons and the dispersion of the chemical shifts for the NH and H–C(β) protons suggest that a stable secondary structure of β-heptapeptide **2c** is present in solution. ROESY Spectra with three different mixing times (50, 100, and 150 ms) were measured, and the resulting NOE cross peaks are presented in *Table 6*.

For the NH protons of residues $i = 1$, 2, and 4, weak-to-medium NOEs to one of the diastereotopic C(β)-protons (denoted H_{ax} of residues ($i + 2$) and ($i + 3$)) are observed (see *Table 5*). The C(α)-protons of each of the residues $i = 2$, 3, and 4 exhibit a medium-to-strong NOE to one of the C(β)-protons (H_{ax}) of residue ($i + 3$). This NOE pattern is typical for a 3_1-helical conformation as reported before [4][5][9]. NOEs between NH_i and $NH_{(i+1)}$ of residues $i = 1$, 2, 4, 5, and 6 further confirm a helical secondary structure, because such a pattern is observed only for turn or helical structures [31].

These NOEs were used as restraints in *simulated annealing* [32] using the AMBER* force field and molecular model [33]. Fifty starting structures were generated by unrestrained molecular-dynamics (MD) [34] simulations at 700 K. A total of 57 NOE cross peaks were classified in three distance categories with upper bound distance limits of 3.0, 3.5, and 4.5 Å for strong, medium, and weak NOEs, respectively. Using these restraints, each of the fifty starting structures was subjected to simulated annealing from 700 K to 1 K and then minimized. The twenty structures lowest in energy were used in a final simulated annealing procedure (300 K → 1 K) with 13 additional torsion angle restraints. The structural bundle consisting of the ten conformers lowest in energy is depicted in *Fig. 6*.

Clearly, the β^2-peptide **2c** (consisting of (2*R*)-residues) forms a right-handed 3_1-helical structure with H-bonds from N_iH to $C_{(i+2)}O$ that is very well-defined from residues 1 to 6 whereas the C-terminal residue is rather mobile.

[21]) *Cf. Eqn. 1* in the *Exper. Part.*

Table 6. *Weak* (w, 4.5 Å), *Medium* (m, 3.5 Å), *and Strong* (s, 3.0 Å) *NOEs Observed in the ROESY NMR Spectrum of β-Heptapeptide* **2c** *in* CD_3OH

Residue	H-Atom	Residue	H-Atom	NOE	Residue	H-Atom	Residue	H-Atom	NOE	Residue	H-Atom	Residue	H-Atom	NOE
1	α	4	βax	s	3	NH	2	Me	m	5	NH	5	βlat	m
1	βax/lat	4	βax	w	3	NH	3	α	s	5	NH	5	Me	w
1	NH	1	α	s	3	NH	5	βax	m	5	NH	5	β′-CH	w
1	NH	1	βax/lat	s	3	NH	5	Me	w	5	NH	6	NH	w
1	NH	2	NH	w	3	NH	6	βax	m	5	NH	7	βlat	w
1	NH	3	βax	m	4	α	7	βax	m	5	NH	7	βax	w
1	NH	4	βax	w	4	α	7	βlat	m	6	NH	5	α	s
2	α	5	β	s	4	β-Me	7	βax	m	6	NH	5	β′-CH	w
2	α	5	β′-CH	m	4	NH	3	α	s	6	NH	5	Me	m
2	NH	1	γ-Me	m	4	NH	3	β′-CH*H*	w	6	NH	5	β′-CH	m
2	NH	1	β′	?	4	NH	3	γ′-CH	m	6	NH	6	Me	w
2	NH	1	α	s	4	NH	4	α	s	6	NH	6	α	s
2	NH	1	β′-CH	m	4	NH	4	β-Me	m	6	NH	6	βlat	m
2	NH	2	β	m	4	NH	5	NH	w	6	NH	7	NH	w
2	NH	2	α	s	4	NH	6	βax	m	7	NH	6	α	s
2	NH	3	NH	w	4	NH	7	βax	w	7	NH	6	βlat	m
2	NH	4	βax	m	4	NH	7	βlat	w	7	NH	6	βax	w
2	NH	5	βax	m	5	NH	4	α-Me	m	7	NH	6	Me	m
3	α	6	βax	s	5	NH	4	α	s	7	NH	7	β′-CH_2	w
3	NH	2	α	s	5	NH	5	α	m	7	NH	7	α	s

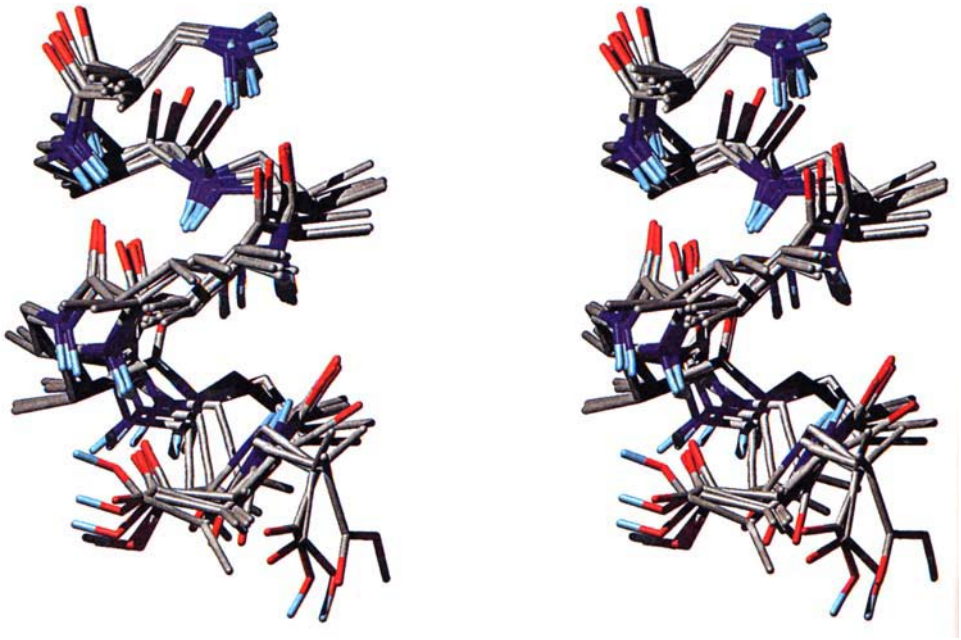


Fig. 6. *NMR Solution structure* (stereo side view) *of the β²-heptapeptide* **2c**. This peptide is present in CD_3OH as right-handed 3_1 helix, as can be seen from the bundle of the 10 conformers lowest in energy derived from restrained simulated annealing. For clarity, the side chains have been substituted by Me, and C-bound H-atoms have been omitted. The figure was generated by MolMol [35] and raytraced by POV-Ray.

4.2.2.2. *The β-Hexapeptide* **7c** *with Side Chains in Both the 2- and 3-Position.* It was investigated in CD_3OH using the same methods as described above (so far, only $\beta^{2,3}$-peptides built from *trans*-2-aminocyclohexane- and *trans*-2-aminocyclopentanecarboxylic acids, *i.e.*, with conformationally fixed C(α)–C(β) bond, have been investigated [9]). The ^1H and ^{13}C chemical shifts as well as the ^3J coupling constants for **7c** are shown in *Tables 7* and *8*. The dispersion of the chemical shifts for the NH, H–C(α), and H–C(β) protons is again very large. Together with the coupling constants for the backbone, they suggest a secondary structure of great stability. The small coupling constants (2.7 and 2.9 Hz) between the γ-CH and β-CH protons of the $\beta^{2,3}$-valine residue, however, show that the side chain is not freely rotating around the C(β)–C(γ) bond. This is an indication for steric hindrance between the Me_2CH side chain and the adjacent Me group which could destabilize the secondary structure. A 2D-NMR investigation of this hexapeptide was performed, and the resulting NOEs for the backbone atoms are collected in *Table 9*. These NOEs for **7c** are weaker in intensity compared to **2c**. Due to the substituents in 2- and 3-position of the β-amino acids in **7c**, fewer interresidual NOEs were observed than with the β-heptapeptide **2c**. However, the overall pattern of NOEs observed with **7c** is in full agreement with a 3_1-helix conformation of high population in methanol at room temperature.

The experimental data were used as restraints for the structure determination using simulated annealing and the resulting structural bundle is depicted in *Fig. 7*. The 3_1 helix is very well-defined for the residues 2 to 6, with slightly greater structural variance for

Table 7. *^{1}H-NMR Chemical Shifts* (CD_3OH) *and Coupling Constants for the Hexapeptide* **7c**. Diastereotopic CH_2 protons and Me groups (on valine and leucine side chains) are labeled with primes or in italics in the order of decreasing ^{1}H-chemical shifts.

Residue	$\beta^{2,3}$-HVal[1]	$\beta^{2,3}$-HAla[2]	$\beta^{2,3}$-HLeu[3]	$\beta^{2,3}$-HVal[4]	$\beta^{2,3}$-HAla[5]	$\beta^{2,3}$-HLeu[6]
NH, NH_3	7.52 (br.)	8.62 (J(NH,αax) = 9.3)	8.5 (J(NH,αax) = 9.6	7.42 (J(NH,αax) = 10.0)	7.33 (J(NH,αax) = 9.2)	7.96 (J(NH,αax) = 9.9)
H_{ax}–C(β)	3.39 (J(βax,αax) = 10.8)	4.19 (J(βax,αax) = 11.1)	3.99 (J(βax,αax) = 10.6)	4.03 (J(βax,αax) = 11.3)	4.13 (J(βax,αax) = 10.9)	4.13 (J(βax,αax) = 9.3)
H_{ax}–C(α)	2.90 (J(α,β′-CH_3) = 6.9)	3.04 (J(α,β′-CH_3) = 6.9)	2.69 (J(α,β′-CH_3) = 7.0)	2.49 (J(α,β′-CH_3) = 6.9	2.3 (J(α,β′-CH_3) = 6.9)	2.55 (J(α,β′-CH_3) = 6.7)
α-CH_3	1.18	1.16	1.19	1.10	1.18	1.22
H–C(γ)	2.22 (J(γ-CH,β-CH) = 2.7)	– –	– –	1.93 (J(γ-CH,β-CH) = 2.9)	–	– –
δ-CH_3	1.16 (J = 7.0)	–	–	0.88 (J = 6.9)	–	–
δ-CH_3′	1.09 (J = 7.0)	–	–	0.85 (J = 7.0)	–	–
β-CH_3	–	1.19 (J = 7.0)	–	–	1.10 (J = 7.0)	–
γ-C*H*H	–	–	1.34	–	–	1.40
γ-CH*H*	–	–	1.30	–	–	1.38
δ-CH	–	–	1.55	–	–	1.62
ε-CH_3	–	–	0.96 (J = 6.3)	–	–	0.94 (J = 6.5)
ε-CH_3′	–	–	0.87 (J = 7.0)	–	–	0.94 (J = 6.7)

Table 8. *^{13}C-NMR Chemical Shifts* (CD_3OH) *for the β-Hexapeptide* **7c**. Diastereotopic Me groups (on valine and leucine side chains) are labeled with primes in the order of decreasing chemical shifts.

Residue	$\beta^{2,3}$-HVal[1]	$\beta^{2,3}$-HAla[2]	$\beta^{2,3}$-HLeu[3]	$\beta^{2,3}$-HVal[4]	$\beta^{2,3}$-HAla[5]	$\beta^{2,3}$-HLeu[6]
C(α)	43.3	47.6	47.2	44	47.7	47.1
C(β)	61.1	48	49.9	55.7	48	50.7
$CH_3(\beta')$	15.1	16.9	17.9	16.5	17.5	15.4
$CH_2(\gamma)$	–	–	44.4	–	–	43.2
CH(γ)	29.6	–	–	27.6	–	–
$CH_3(\gamma)$	–	18.5	–	–	18.3	–
$CH_3(\delta)$	20.1	–	–	24.4	–	–
$CH_3'(\delta)$	15.7	–	–	20.8	–	–
CH(δ)	–	–	25.9	–	–	25.5
$CH_3(\varepsilon)$	–	–	22.2	–	–	24.1
$CH_3'(\varepsilon)$	–	–	15.7	–	–	22.4
C=O	174.9	177.9	177.3	175.6	175.7	178.4

Table 9. *Weak* (w, 4.5 Å), *Medium* (m, 3.5 Å), *and Strong* (s, 3.0 Å) *NOEs Observed for the Backbone in the ROESY NMR Spectrum of β-Hexapeptide* **7c** *in CD_3OH*

Residue	H-Atom	Residue	H-Atom	NOE	Residue	H-Atom	Residue	H-Atom	NOE
1	α	4	β	s	3	NH	2	α	s
1	NH	1	α	m	3	NH	3	α	s
1	NH	2	NH	w	3	NH	4	NH	w
1	NH	2	β	w	3	NH	6	β	m
1	NH	3	β	w	4	NH	3	α	s
1	NH	4	β	w	4	NH	4	α	s
2	α	5	β	s	4	NH	6	β	w
2	NH	1	β	w	5	NH	4	α	s
2	NH	1	α	s	5	NH	5	α	s
2	NH	2	α	s	5	NH	6	NH	w
2	NH	4	β	m	6	NH	5	α	s
2	NH	5	β	m	6	NH	6	α	*m*
3	α	6	β	m					

the N-terminal residue. Bond lengths were found to range for N(1)–H···O=C(3) from 1.8 to 2.0 Å, for N(2)–H···O=C(4) from 1.6 to 1.9 Å, and for N(3)–H···O=C(5) from 1.8 to 2.1 Å, while no H-bond results from N(4)–H to the terminal carboxy O-atom.

From the top view, the 'steric protection' of the peptide backbone by the many (hydrophobic) substituents is clearly evident. This 'protection' is probably causing the very slow exchange of the NH protons of the β-hexapeptide **7c** (see *Sect. 4.2.1*). On the other hand and as mentioned above, this steric crowding may be a destabilizing contribution (unwinding of the helix). A consequence of side-chain repulsion is visible in the top view of the 3_1 helix (right side of *Fig. 7*); there is a twist so that precise juxtaposition of Me_2CH and Me_2CHCH_2 is avoided. Another contribution to this deviation from the ideal 3_1-helical geometry may come from the Me groups of the Me_2CH side chains of residues 1 and 4 that are located in a plane approximately parallel to the helix axis.

4.2.2.3. *Solution Structures of the β-Hexapeptide* **4** *with Alternating β^2/β^3-Amino-Acid Residues*. From the CD spectra of β-peptide **4** with (**a**) and without (**c**, **d**) protected

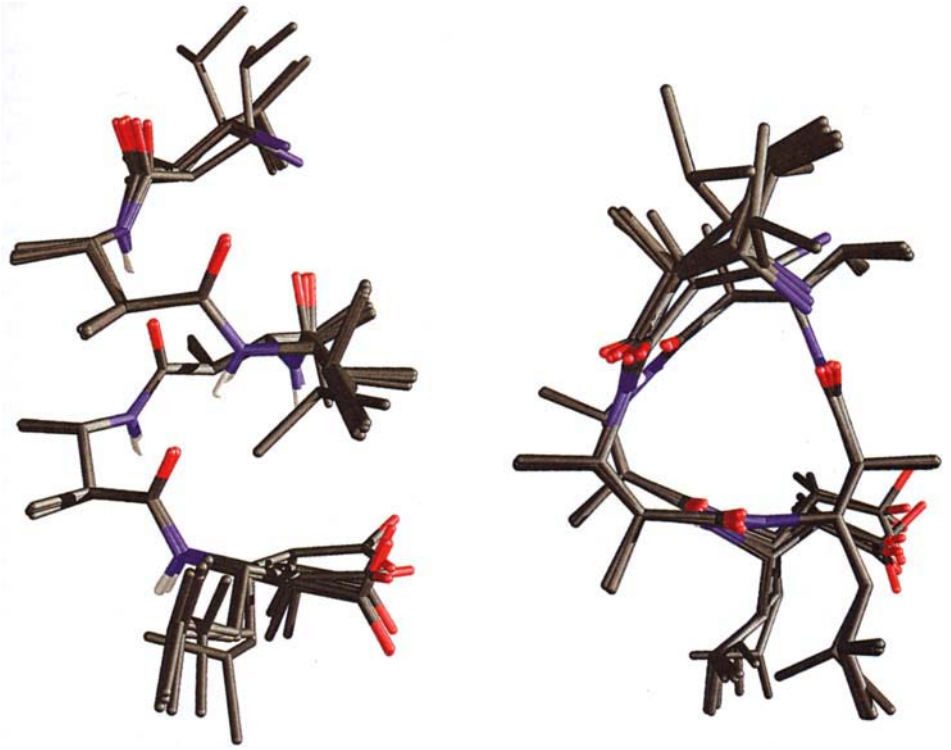


Fig. 7. *NMR Solution structure* (side view and top view) *of* $\beta^{2,3}$*-hexapeptide* **7c** *in* CD_3OH. Bundle of the six conformers lowest in energy determined by restrained simulated annealing. All C-bound H-atoms have been omitted for clarity. The figure was generated by Raster3D [36].

termini, we expected to detect a new type of secondary structure by NMR measurements. The first analysis was done in the basic solvent pyridine, the second one in methanol, both with the unprotected β-peptide.

4.2.2.3.1. *NMR Structure Determination of* **4d** *in* (D_5)*Pyridine Solution*[22]). A total of 40 distance restraints (17 intraresidual, 16 sequential, and 7 where $|i-j| = 2$) have been extracted from ROESY spectra (*Table 10*). The restraints were conservatively classified into upper distance limits of 2.8, 3.5, and 4.5 Å for strong, medium, and weak cross-peak intensities, respectively. During structure determination, stereospecific assignments of some CH_2 protons (the chemical shifts in the ^{1}H-NMR spectrum are collected in *Table 11*) was possible by comparing measured ^3J coupling constants with the preferred conformations observed in an ensemble of low-energy structures. A set of 20 structures was calculated, using simulated annealing protocols in X-PLOR. All structures converged to essentially the same fold with no violations greater than 0.1 Å to the experimentally derived distance restraints. A set of 16 structures with lowest overall energies was selected for being representative of the solution conformation of β-peptide **4d** in

[22]) In this solvent, the N-terminus may probably be assumed to be non-protonated.

Table 10. *Interproton Distance Restraints for the β-Hexapeptide* **4d** *in (D5)Pyridine Determined from ROESY Spectra*

Residue *i*	H-Atom	Residue *j*	H-Atom	NOE
1	H–C(α)	2	NH	s
1	2H–C(β)	2	NH	w
1	2H–C(β)	3	H–C(α)	w
2	NH	2	H_{Re}–C(α)	m
2	NH	2	Me(γ)	s
2	H–C(β)	3	NH	m
2	H_{Re}–C(α)	3	NH	w
2	H_{Si}–C(α)	3	NH	s
2	H_{Si}–C(α)	3	H_{Si}–C(β)	w
2	H–C(β)	4	NH	s
2	H–C(β)	4	H_{Re}–C(α)	s
3	NH	3	H_{Si}–C(β)	m
3	NH	3	H_{Re}–C(β)	s
3	NH	3	H–C(α)	m
3	NH	4	H_{Re}–C(α)	w
3	NH	4	H–C(β)	w
3	H–C(α)	4	NH	s
3	H_{Re}–C(β)	4	NH	m
3	NH	5	H_{Si}–C(β)	w
4	NH	4	H–C(β)	m
4	NH	4	H_{Re}–C(α)	s
4	NH	4	H_{Si}–C(α)	w
4	NH	4	H–C(γ)	m
4	H–C(β)	5	NH	m
4	H_{Re}–C(α)	5	NH	m
4	H_{Si}–C(α)	5	NH	s
4	H_{Si}–C(α)	6	NH	w
4	H–C(β)	6	NH	m
4	H–C(β)	6	2H–C(α)	w
5	NH	5	H_{Re}–C(β)	s
5	NH	5	H_{Si}–C(β)	m
5	NH	5	H–C(α)	m
5	H_{Re}–C(β)	6	NH	w
5	H_{Si}–C(β)	6	NH	w
5	H–C(α)	6	NH	s
6	NH	6	H–C(β)	m
6	NH	6	2H–C(α)	w
6	NH	6	H–C(δ)	m
6	NH	6	2H–C(γ)	w
6	H–C(β)	6	H–C(δ)	m

Table 11. *^{1}H-NMR Chemical Shifts* ((D_5)pyridine) *for the β-Hexapeptide* **4d** *at 23°*

Residue	β^2-HVal[1]	β^3-HAla[2]	β^2-HLeu[3]
NH,NH_3	not available	10.14	9.04
H–C(β)[1])	3.64, 3.25 (2 H–C(β))	4.86 (H–C(β))	3.70 (H_{Si}–C(β)); 2.89 (H_{Si}–C(β))
H–C(α)[1])	3.43 (H–C(α))	2.80 (H_{Si}–C(α)); 2.51 (H_{Re}–C(α))	2.95 (H–C(α))
Side-chain Protons[1])	1.94 (H–C(γ)); 0.96, 0.86 (2 Me(δ))	1.43 Me(γ)	1.74, 0.89 (2 H–C(γ)); 1.64 (H–C(δ)); 0.94 (2 Me(ε))
Residue	β^3-HVal[4]	β^2-HAla[5]	β^3-HLeu[6]
NH,NH_3	8.92	8.88	8.81
H–C(β)[1])	4.62 (H–C(β))	3.88 (H_{Si}–C(β)); 3.07 (H_{Re}–C(β))	4.90 (H–C(β))
H–C(α)[1])	2.69 (H_{Si}–C(α)); 2.28 (H_{Re}–C(α))	2.80 (H–C(α))	2.97, 2.77 (2 H–C(α))
Side-chain Protons[1])	1.80 (H–C(γ)); 1.00, 0.96 (2 Me(δ))	1.20 (Me(γ))	1.74, 1.50 (2 H–C(γ)); 1.97 (H–C(δ)); 0.93, 0.93 (2 Me(ε))

[1]) For simplicity, the designation of side chains starts with C(γ) for all residues, including β^2-amino acids.

Table 12. *Statistics of the X-PLOR Structure Calculation for* **4d** *in* (D_5)*Pyridine*

1) Final energies and standard deviations [kcal · mol^{-1}] *for 16 calculated structures*

F_{total}	F_{bond}	F_{angle}	$F_{improper}$	F_{repel} [a])	F_{NOE} [b])
0.18 ± 0.13	0.01 ± 0.01	0.09 ± 0.07	0.04 ± 0.01	0.003 ± 0.006	0.04 ± 0.05

[a]) The quartic *van der Waals* term was calculated with a force constant of 4 kcal · mol^{-4} with the *van der Waals* radii set to 0.75 times the standard value used on the CHARMm empirical energy function.

[b]) The final values of the square-well NOE potential is calculated with a force constant of 50 kcal · mol^{-1}.

2) Atomic root mean-square differences [Å]

	Backbone atoms	Heavy atoms
Residues 2–5	0.4	0.8
All residues	0.9	1.5

(D_5)pyridine solution (*Fig. 8*). The final energies of the selected structures are summarized in *Table 12*.

The structure can be described as well-defined for the backbone of residues 2 to 5 with an atomic root mean-square deviation (after best-fit superposition) of 0.4 Å. The side

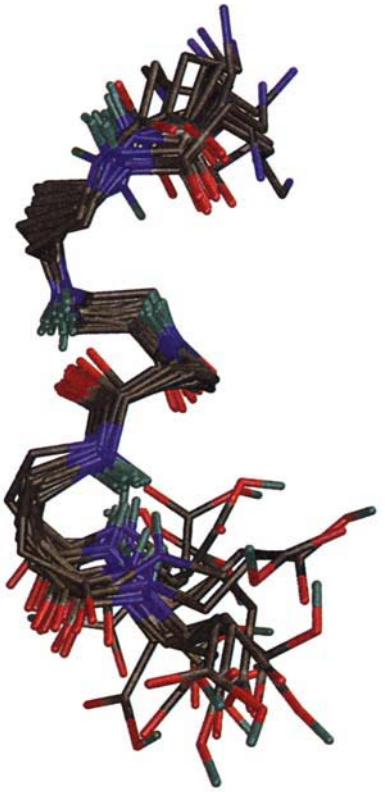


Fig. 8. *NMR Solution structure of 'mixed' β-hexapeptide* **4c** *in* (D_5)*pyridine*. Side view of the 16 structures lowest in energy calculated by restrained simulated annealing. For clarity, the side chains have been omitted. The figure was generated by Raster3D [36].

chains show considerably higher disorder, and this was also found for the terminal residues of the hexapeptide.

We note that all the calculated structures display close proximity of the NH atom of residue 3 and the C=O group of residue 4 suitable for the formation of a central H-bond. The formation of additional H-bonds between the C=O groups of residue 1 and 4, and NH of residue 3 and 6, respectively, is much less pronounced.

4.2.2.3.2. *The NMR Structure Determination of* **4c** *in* CD_3OH *Solution.* The new types of secondary structures discovered for the β-peptide **4** in pyridine (by NMR) and suggested by an unusual CD pattern in methanol may have nothing in common! It was, therefore, of outmost importance to perform an NMR investigation in CD_3OH of the β-hexapeptide **4c**. Since all the β-amino acids of **4c** are unique in their constitution, a COSY experiment led to the assignment of all resonances in the ^1H spectrum as well as to the determination of the sequence. The ^1H chemical shifts and coupling constants derived from the ^1H spectrum are collected in *Table 13*. It can be concluded from the dispersion of the chemical shifts and the very large ^3J coupling constants (*i.e.*, 11 to 12 Hz for ^3J(H–C(α),H–C(β))) that at least one stable secondary structure is populated in methanol at room temperature. Some of the coupling constants differ from those found for 3_1 helices, *e.g.*, the ^3J(NH,CH_2(β)) of residue 3, which are both rather small (5.3 and 7.0 Hz). Therefore, ROESY experiments were performed in order to gain more information, and the resulting NOEs are presented in *Table 14*. Again, some of the NOEs are not compatible with a 3_1-helical conformation at all: from H–C(β) of residue 2 to H–C(α) of residue 4 as well as from H–C(β) to C(α) of residue 6. This *i* to (*i* + 2) pattern is also found for the strong NOEs from H–C(β) of residue 2 to the NH of residue 4 and from H–C(β) of residue 4 to the NH of residue 6. However, some *i* to (*i* + 3) NOEs present in CD_3OH solution (*e.g.*, between α of residue 1 and β of residue 4, or between H–C(α) of residue 3 and H–C(β) of residue 6) were not found in (D_5)pyridine solution. These NOEs remind of the typical *i* to (*i* + 3) pattern of the 3_1-helical conformation. From these facts, it was not clear yet whether an ensemble of different conformers, *e.g.*, a 3_1 helix and a new type of secondary structure, is populated, or whether the data are in agreement with a single predominant conformation, such as the 12/10/12 helix found in pyridine or even a new kind of secondary structure. Since NOEs and coupling constants represent the averaged ensemble, it is not possible to distinguish between these three cases *a priori*.

The structure determination was performed using X-PLOR 3.851 with the assumption that one NOE corresponds to one distance range; thus, time-averaging of several conformational types was not taken into account. The resonances of the diastereotopic CH_2 protons of residues 2, 3, and 4 were assigned such that the large ^3J value to the adjacent CH results from coupling to H_{Re}. The 34 NOEs shown in *Table 14* were transformed into distance restraints with 3.0 Å, 3.5 Å, and 4.5 Å for strong, medium and weak cross peak volumes, respectively, as upper bound distance restraints and their respective *van der Waals* radii as the lower bound distance restraints. Furthermore, nine dihedral angle restraints extracted from ^3J coupling constants *via* the *Karplus* equation were used in the *ab initio* simulated annealing protocol of X-PLOR 3.851. The calculation converged well, and the resulting structural bundles are shown in *Figs. 9* and *10*. The calculated structures fall into two types of conformations, which are depicted in *Fig. 9,a*. The structure in green (denoted $\mathbf{K_L}$) is calculated to be by *ca.* 30 kcal/mol lower in energy

Table 13. *^{1}H-NMR Chemical Shifts* (CD_3OH) *and Coupling Constants for the β-Hexapeptide* **4c**. Diastereotopic protons and Me groups are labeled with primes or in italics in the order of decreasing ^{1}H-chemical shifts. Diastereotopic CH_2 protons of residues 2 and 4 were stereospecifically assigned and are denoted H_{Re} and H_{Si}.

Residue	β^2-HVal[1]	β^3-HAla[2]	β^2-HLeu[3]	β^3-HVal[4]	β^2-HAla[5]	β^3-HLeu[6]
NH, NH_3	7.84–7.82 (br.)	8.34 (J(NH,β) = 8.4)	8.46 (J(NH,β) = 7.0; J(NH,β′) = 5.3)	8.19 (J(NH,β) = 9.5)	7.84 (J(NH,β) = 5.7; J(NH,β′) = 5.7)	8.24 (J(NH,βax) = 8.4)
H–C(β)	3.07–2.97 (br.)	4.41	3.30	4.15	3.28	4.27
H′–C(β)	3.07–2.97 (br.)	–	2.82	–	2.98	–
^2J(β-CH,β-CH′)	–	–	13.2	–	15[1])	–
^3J(β-CH,α-CH)	–	3.0	4.0	3.0	n/a	5.0
^3J(β-CH,α-CH′)	–	11.5	–	12.0	–	9.5
^3J(β-CH′,α-CH)	–	–	11.0	–	10[a])	–
H–C(α)	2.30	2.49	2.60	2.43	2.48	2.45
H′–C(α)	–	2.09	–	2.06	–	2.36
^2J(α-CH,α-CH′)	–	13.1	–	13.5	–	15.0
	δ(H–C(β′)) = 1.75	–	δ(H–C(γ)) = 1.60	δ(H–C(γ)) = 1.70	–	δ(H–C(δ)) = 1.46
	–	–	δ(H–C(β′)) = 1.52	–	–	δ(H–C(γ)) = 1.39
	–	–	δ(H′–C(β′)) = 1.49	–	–	δ(H′–C(γ)) = 1.22
	δ(CH'_3(γ)) = 0.91	δ(CH_3(γ)) = 1.15	δ(CH_3(γ)) = 0.83	δ(CH_3(δ)) = 0.85	δ(CH_3(β′)) = 0.96	δ(CH_3(ε)) = 0.86
	δ(CH'_3(γ)) = 0.86	–	δ(CH'_3(γ)) = 0.83	δ(CH'_3(δ)) = 0.82	–	δ(CH'_3(ε)) = 0.81

[a]) Derived from DQF.COSY experiments.

Table 14. *Weak* (w, 4.5 Å), *Medium* (m, 3.5 Å), *and Strong* (s, 3.0 Å) *NOEs Observed for the Conformationally Relevant Protons in the ROESY NMR Spectrum of β-Hexapeptide* **4c** *in* CD_3OH. Diastereotopic CH_2 protons of residues 2 and 4 were stereospecifically assigned and are denoted H_{Re} and H_{Si}.

Residue *i*	H-Atom	Residue *j*	H-Atom	NOE	Residue *i*	H-Atom	Residue *j*	H-Atom	NOE
1	β′, β	1	Me_2CH	m	3	β	3	α	s
1	β′, β	3	α	w	3	α	6	β	w
1	α	4	β	m	4	NH	2	β	s
2	NH	1	α	s	4	NH	3	β′	w
2	NH	1	β′, β	w	4	NH	3	α	s
2	NH	1	Me_2CH	w	4	NH	4	β	s
2	NH	2	β	s	4	NH	4	α′ (H_{Re})	m
2	NH	2	α/α′	w	4	NH	4	Me_2CH	m
2	β	2	α/α′	s	4	β	4	Me_2CH	s
2	β	4	α′ (H_{Re})	s	4	β	6	α	w
3	NH	2	β	s	5	β	5	β′	s
3	NH	2	α/α′	m	6	NH	4	β	s
3	NH	3	β′	m	6	NH	4	α (H_{Si})	w
3	NH	3	α	m	6	NH	5	α	s
3	NH	5	α	w	6	NH	5	β′	w
3	β′	1	α	w	6	NH	6	β	s
3	β′	3	β	s	6	β	6	γ-CH_2	w

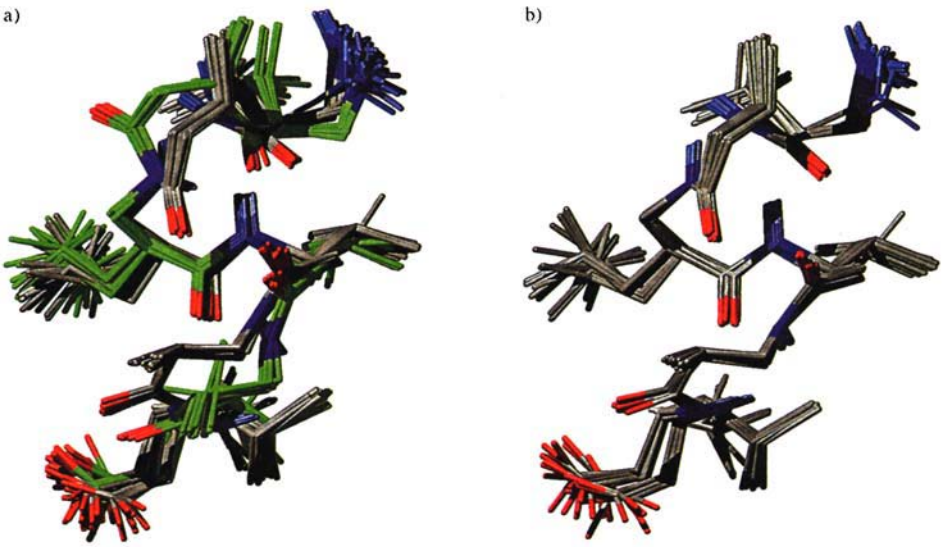


Fig. 9. *NMR Solution structure of 'mixed' β-hexapeptide* **4c** *in* CD_3OH. *a*) Side view of a superposition of the conformers $\mathbf{K_L}$ (green) and $\mathbf{K_H}$ (gray), as determined from NMR measurements and restrained simulated annealing. The conformer $\mathbf{K_L}$ was found to be lower in energy, thus indicating a possible preference of this conformer. All C-bound H-atoms have been omitted for clarity. *b*) Side view of conformer $\mathbf{K_H}$, which is by *ca.* 30 kcal/mol higher in energy. Note that three C=O groups are pointing towards each other, indicating possible *van der Waals* and electrostatic disturbances. All C-bound H-atoms have been omitted for clarity. The figures were generated by MolMol [35] and raytraced by POV-Ray.

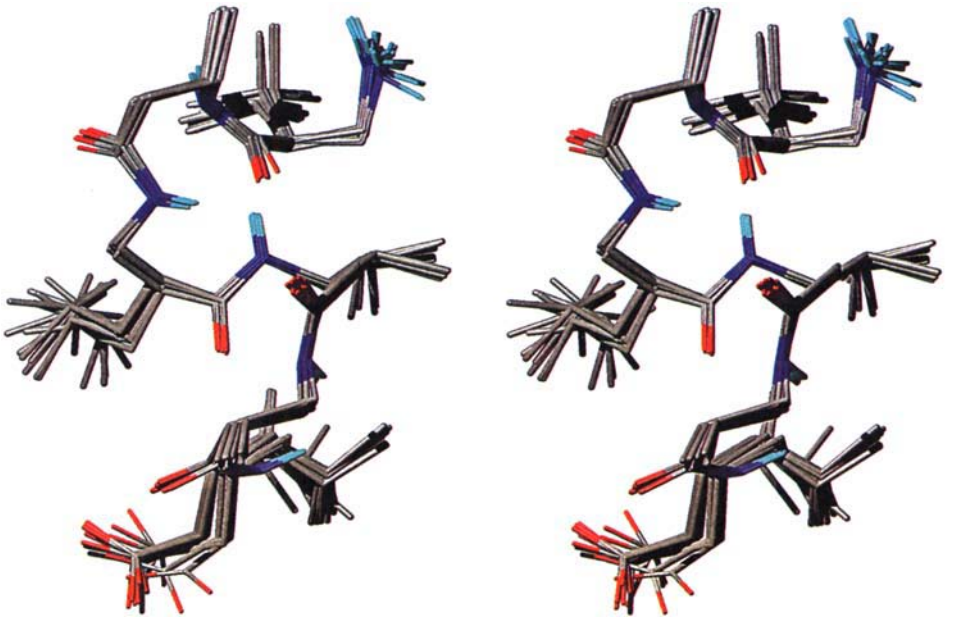


Fig. 10. *NMR Solution structure* (stereo side view) *of conformer* $\mathbf{K_L}$ *of* **4c**. This bundle of structures is the lowest in energy according to X-PLOR calculations, indicating that it is highly populated in CD_3OH. All C-bound H-atoms have been omitted for clarity. The figures were generated by MolMol [35] and ray-traced by POV-Ray.

than the gray one (denoted $\mathbf{K_H}$). However, the *entropy* of these structures was not taken into account; therefore, energy terms cannot be used to discuss which of the conformers might be more stable.

Conformer $\mathbf{K_L}$ (*Fig. 9,a*, green, and *Fig. 10*), is characterized by an N-terminal twelve membered H-bonding ring with the H-bond from C=O of residue 1 to NH of residue 4 (H-bond lengths range from 1.1 to 1.4 Å) and a central ten-membered H-bonded ring with a C=O···HN H-bond from residue 3 to 4. The pattern strongly resembles the structure of **4d** in (D_5)pyridine solution. This is particularly remarkable in view of the fact that no H-bonds were explicitly considered or defined in the calculation, and that only the experimentally accessible parameters were used. The C-terminal twelve-membered H-bonded ring is not present, and the amide bond between residues 5 and 6 is orthogonal to the main axis.

Conformer $\mathbf{K_H}$ (*Fig. 9,b*) differs from conformer $\mathbf{K_L}$ in a small deviation of the backbone C-atoms of residues 2 and 5, and in a major deviation in which the amide bond between alanine 2 and leucine 3 is turned around so that its C=O group directs toward the C-terminus. This implies that the central ten-membered H-bonding ring is absent, and that three C=O groups of residues 2, 3, and 4 point in the same direction. The resulting electrostatic and *van der Waals* repulsion potential suggests that $\mathbf{K_H}$ is less populated at room temperature than conformer $\mathbf{K_L}$. There is, however, no experimental evidence for a smaller stability of $\mathbf{K_H}$, since neither conformer involves NOE violations larger than 0.3 Å. More elaborate MD simulations with explicit solvent molecules included (*cf.* [6][37]) may answer the question as to whether several conformations co-exist.

4.2.2.3.3. *Unrestrained Molecular-Dynamics Simulation of* **4**. To generate a low-energy model structure of the hexapeptide **4**, a long, 10-ns MD simulation without any experimental restraints at 50 K was performed using the AMBER* molecular model and force field[23]). The length of the simulation ensures that enough of the conformationally available space is sampled. A conformation from the (D_5)pyridine structure calculations was used as starting point, and implicit water was used to avoid extreme electrostatic interaction. Twenty structures along the trajectory were sampled and minimized. Since there is a RMSD of only 0.11 Å for all heavy atoms, the resulting bundle must represent a minimum on the energy hypersurface. A mean structure was calculated and is shown in *Fig. 11*. This structure is consistent with the NMR-derived data from (D_5)pyridine solution and contains two terminal twelve-membered and a central ten-membered H-bonding ring. It is interesting to note that the peptide bonds with no adjacent substituents (between residues 2 and 3, as well as 4 and 5) form the central H-bond. These amide groups seem to be more flexible and thus prefer nearest-neighbor H-bonding. The other, conformationally more restricted amide groups with two neighboring substituents (between residues 1 and 2, 3 and 4, and 5 and 6) are forming H-bonds among themselves and favor interresidual (i to $i + 3$) H-bonding. This correlation between steric hindrance of the amide group and H-bond formation is especially noteworthy, because we have

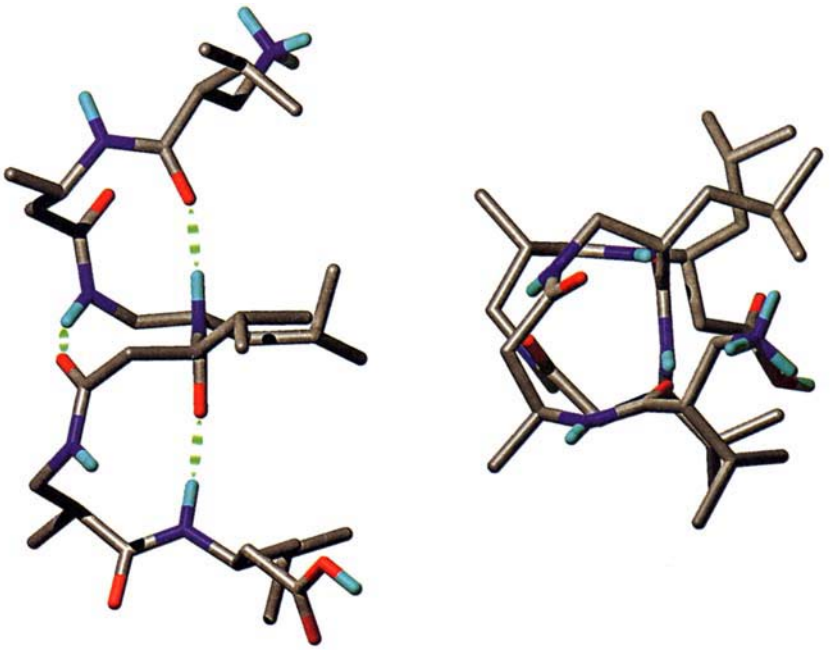


Fig. 11. *Side and top view of the low-energy model of β-peptide* **4**. Obtained by a long, 10-ns MD simulation without any experimental restraints in water at 50 K using the AMBER* molecular model and force field. All C-bound H-atoms have been omitted for clarity. The figures were generated by MolMol [35] and raytraced by POV-Ray.

[23]) The AMBER force field [33a,b] was improved by *McDonald* and *Still* [33c] for application to small peptides [33d].

previously observed that a protected β^3-heptapeptide with an unsubstituted (β-HGly, *i.e.*, 3-aminopropionic acid) residue in the central position does not show the CD pattern indicative of a 3_1 helix[24]) [5].

It is evident from the top view in *Fig. 11* that hydrophobic effects play a major role in stabilizing such a conformation. The valine and leucine side chains are in direct juxtaposition, while the alanine Me groups are on the other side, so that this helical structure is polar, with different hydrophobicity on the two faces.

5. Conclusion. – The following conclusions from and (partially speculative) interpretations of the results described in the previous sections are considered important.

i) In the series of β-peptides studied by us, a β^2-hexapeptide forms a less stable 3_1 helix as compared to a β^3-hexapeptide with the same side chains. An additional $\beta^{2,3}$-HAla(α-Me) residue in the central position of the corresponding heptapeptides reduces this stability difference (*Fig. 4, a*, *Sect. 4.2.2.1*, and previous publications [4][5]). This difference in stability might be interpreted as resulting from smaller steric hindrance between NH and a neighboring substituent (in β^3-residues) as compared to CO and a neighboring R group (in β^2-residues). This could lead to a weakening of the intramolecular H-bonds. Conversely, there might be better solvation by MeOH in the non-folded form of a β^2-peptide.

ii) While the NH/ND exchange rates in the central part of the $\beta^{2,3}$-heptapeptide **7** are the largest of all β-hexa- and β-heptapeptides measured (*Tables 2* and *3*), the intensity of the cross-peaks in its ROESY spectrum is weaker than with the analogous β^3-hexapeptide [4] lacking the α-Me groups (less highly populated helix form). This may be due to a helix destabilization by steric repulsion between side chains, as evident from the dislocation of adjacent turns (*Fig. 7*, top view[25])).

iii) What are the major structural differences between the old (3_1 or 3_{14}) (*M*)-helix (*Figs. 1, a*, *6*, *7*, and [4][5][9a]) and the new (*P*)-helix (*Figs. 8–11* and [1])? First of all, the 3_1 helix has a resulting dipole moment with the positive end at the C- and the negative end at the N-terminus. The new helix consists of a sequence of three H-bonded turns: a central ten-membered ring and two terminal twelve-membered rings (side-view in *Fig. 11*), we designate it a 12/10/12 helix; with its C=O and NH groups pointing alternately up and down along the helix axis, the resulting dipole moment should be near zero. It could well be that the surprising solubility of the mixed β-peptide derivatives (especially those with alternating β^2/β^3 residues) in non-polar aprotic solvents (such as AcOEt) has to do with the non-polar nature of the 12/10/12 helix.

iv) After helix and pleated sheet structures of β-peptides, we have, thus, now identified the third type of secondary structure found in proteins, namely turns; fitting with the larger structural variety of β- as compared to α-peptides, two quite different types of turns have been discovered at once (*Fig. 12*)[26]).

---

[24]) In this case, it might be energetically favorable for the peptide bond with no substituents to form H-bonds other than those fitting in the *i* to (*i* + 2) pattern of the 3_1 helix. This aspect might be interesting in connection with the question, as to when nearest neighbor H-bonding is favored in β-peptides [12b][38] with different substitution pattern of their constituent β-amino-acid residues.

[25]) The cyclic structure of the $\beta^{2,3}$-amino-acid residues in *Gellman*'s β-peptide not only locks the conformation around the C(2)–C(3) bond but also prevents the kind of steric crowding present in the helix of **7**.

[26]) The 14-membered H-bonded ring in the 3_1 helix may also be considered a turn. For a discussion of 'turns without H-bond', see Fig. 2 in [5].

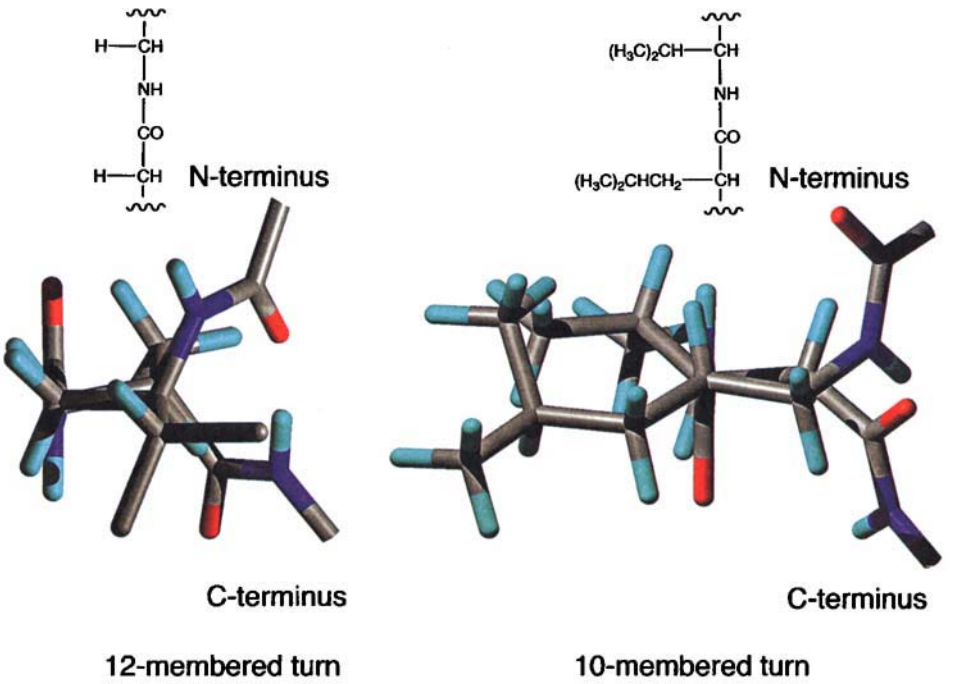


Fig. 12. *Comparison of the two turns found in the 12/10/12 helix, with the projection along the amide plane (CH$_2$–CO–NH–CH$_2$ and CH(CH$_2$–CH(Me)$_2$)–CO–NH–CH(CH(Me)$_2$)).* Note that in the case of the twelve-membered turn the flanking substituents are 'eclipsed', while in the ten-membered turn they are 'staggered'. The figures were generated by MolMol [35] and raytraced by POV-Ray.

v) A comparison of the three dihedral angles Φ, Θ, and Ψ[27]) in the β-amino acids of the two types of helices (*Fig. 13*) reveals that the central ethane bond has a (+)-*synclinal* conformation in all cases (Θ positive), while the CO–N–C(3)–C(2) angle Φ is (–) in all β-amino-acid residues except for the β^2-amino-acid moieties in the 12/10/12 helix where it is (+); the same applies to the angles Ψ.

vi) What causes the mixed β-peptide **4** with alternating β^2- and β^3-amino-acid units to adopt the 12/10/12 rather than the 3_1-helical secondary structure? Inspection of the schematic presentations of the top views of β-hexapeptide 3_1 helices with different substitution patterns (*Fig. 14*; *cf. Figs. 1,a*, and *7*) reveals that the number of directly adjacent aliphatic side chains on residues (*i*) and (*i* + 3) differ from 6 to 0. In fact, no such juxtapositions at *ca.* 5-Å distance occur for the mixed β-peptides **3** and **4**, and those are the ones with a tendency to switch over to the 12/10/12 helical structure (see NMR analysis of **4c** and **4d**, CD pattern of **3a** and **4a**). The fact that the large aliphatic side chains wind up on top of each other in the new helix (*Fig. 11*, top view) would suggest that hydrophobic interactions actually cause the preference for the new helix in these cases.

---

[27]) Using the *Balaram* convention [39].

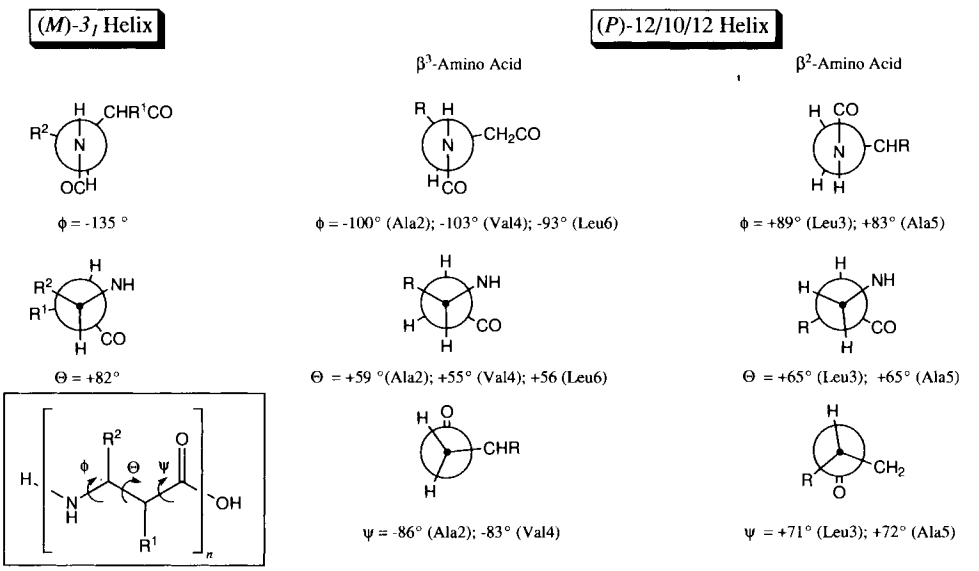


Fig. 13. *A comparison of the dihedral backbone angles for the* 3_1 *helix and the 12/10/12 helix.* The dihedral angles of the 3_1 helix were calculated *via Karplus* equation (see *Exper. Part*) and are based on the backbone *J* coupling constants of the non-terminal residues of peptides **2c**, **7c**, and the β^3-heptapeptide TFA · β^3-HVal-β^3-HAla-β^3-HLeu-β^3-HAla(α-Me)-β^3-HVal-β^3-HAla-β^3-HLeu-OH [5]. The angles of the 12/10/12 helix were taken from the low-energy conformer depicted in *Fig. 11*. It is interesting to note that there are two different sets of dihedral angles in the 12/10/12 helix for the β^2-residues and for the β^3-residues.

vii) The surprising case is the mixed β-peptide **6** which *has* three pairs of adjacent side chains – just like β^3- and β^2-hexapeptides (*e.g.*, **1**) – and still exhibits the CD pattern that we assign to the 12/10/12 helix. It is tempting to speculate that there is a tendency to form the twelve-membered turn (*Fig. 12*, left), and thus the 12/10/12 helix, in the *absence* of substituents on both sides of the amide group of β-peptide residues ($-CH_2-CO-NH-CH_2-$), a common feature of **6** and **4**. This view is supported by the fact that, according to CD data, the β^3-heptapeptide Boc-β^3-HVal-β^3-HAla-β^3-HLeu-β-HGly-β^3-HVal-β^3-HAla-β^3-HLeu-OMe, with a central unsubstituted β-amino acid, and with a $-CH_2-CO-NH-CH_2-$ section, probably also has a 12/10/12 helical structure [5][24])[28]). It is intriguing to notice that – like in α-peptides (Gly) – the unsubstituted β-amino acid (β-HGly) may be turn-inducing.

viii) Why is there a tendency for the 12/10/12-helical structure (new helix) to rearrange to the *14*-helical structure (old helix) upon deprotection of the terminal functional groups? This effect is especially dramatic with the 12mer **5** (*Fig. 4*), but has also been observed with the β-HGly-containing heptapeptide alluded to above[24])[28]). Thus, the hydrophobic interactions (see *vi*) and the effect of amide groups without flanking sub-

[28]) See Fig. 6, discussion on p. 2056/7, and Footnote 10 in [5]. From the CD spectrum ($\Theta = +3.2 \cdot 10^4$ at 204 nm), we would now assign the 12/10/12 helical structure to the Boc-protected methyl ester of this peptide. At the time, we had no explanation and called the peptide a 'black-sheep' case; note that deprotection to the free β-heptapeptide restores the CD pattern (in MeOH) which is typical of the 3_1 helix, just like in some cases described herein.

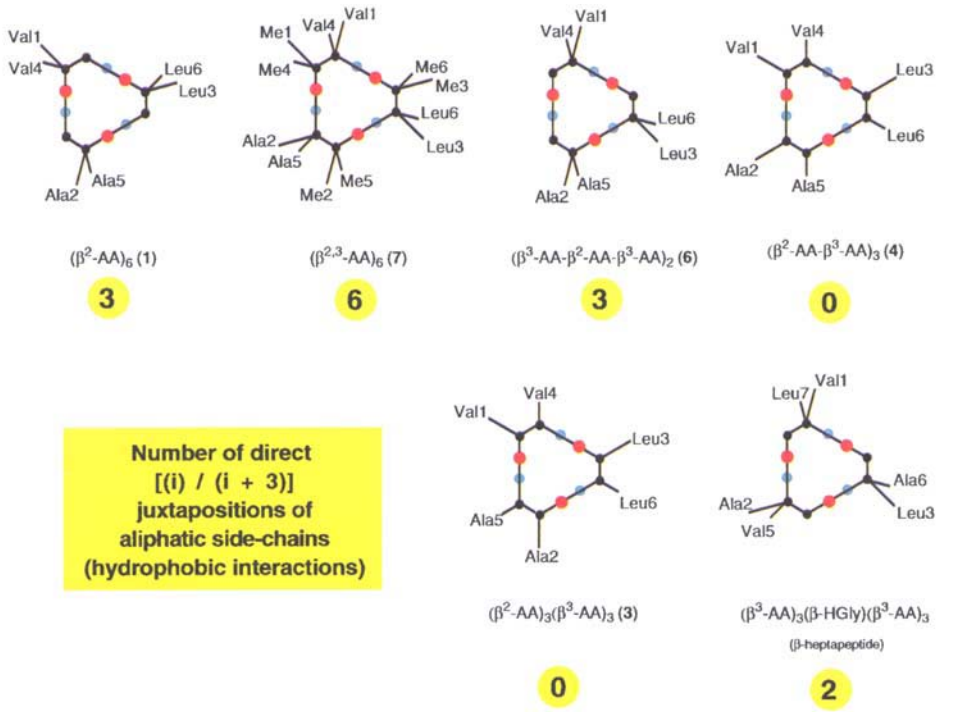


Fig. 14. *Schematic presentations of the top views of β-hexapeptide 3_1 helices with different substitution patterns.* The number of direct [(*i*)/(*i* + 3)] juxtapositions of aliphatic side chains is considered to be important for the stability of the 3_1-helical structure.

stituents (see *vii*), which both appear to stabilize the 12/10/12 helix, are counteracted by the presence of unprotected terminal functional groups, which add stability to the 3_1 helix. The only rationale for this observation, which has occurred to us so far, is the following: the 3_1 helix has a dipole moment with the negative end at the N-terminus, and if the latter were positively charged (NH_3^+)[29]), there would be a *charge-pole stabilization*, lacking in the non-polar 12/10/12 arrangement.

The many subjunctive forms used in *i–viii* of this conclusion clearly show that we are still pupils learning about the rules which govern the structure(s) of β-peptides!

Experimental Part

1. *General.* Abbreviations: Boc_2O (di(*tert*-butyl) dicarbonate), BnOH ($PhCH_2OH$), BzCl (PhCOCl), DCC (1,3-dicyclohexylcarbodiimide), DMAP (4-(dimethylamino)pyridine), DMPU (1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one), 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), β-HXxx (β-homoamino acid)[9]), NMM (*N*-methylmorpholine), TFA (CF_3COOH). THF was freshy distilled over Na/benzophenone under Ar before use. DMF and MeCN were distilled under reduced pressure over CaH_2 and

[29]) So far, we have not conducted a careful systematic investigation of the pH and solvent dependence of the β-peptide CD spectra (see also *Footnote* 15).

stored over 4-Å molecular sieves. Solvents for chromatography and workup were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). Et_3N was distilled from CaH_2 and stored over KOH. $ClCO_2Et$ was distilled and stored at + 4° under Ar. $(i\text{-}Pr)_2NH$ was freshly distilled over CaH_2. LiCl and LiBr were dried at 150° under h.v. for 16 h. All indicated temp. were monitored with an internal thermometer (*Ebro-TTX-690* digital thermometer). Amino-acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. All other reagents were used as received from *Fluka*. The β-amino acids were prepared according to literature procedures [4][5][14]. ***Caution:*** The generation and handling of CH_2N_2 requires special precautions [39]. Reactions carried out with the exclusion of light were performed in flasks completely wrapped in aluminium foil. TLC: *Merck* silica gel *60* F_{254} plates; detection with UV and I_2. FC: *Fluka* silica gel *60* (40–63 μm), at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system (pump type *64*, *EuroChrom 2000* integration package, degaser, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. Circular dichroism (CD) spectra: *Jasco J-710* recording from 190 to 250 nm at r.t.; 1-mm rectangular cell; average of five scans, corrected for the baseline; peptide concentration 0.2 mM in MeOH; molar ellipticity Θ in deg · cm^2 · dmol^{-1} (λ in nm); smoothing by *Jasco* software. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz), *ARX 300* (^{1}H: 300 MHz), *Varian Gemini 300* (^{1}H: 300 MHz, ^{13}C: 75 MHz), or *Varian Gemini 200* (^{1}H: 200 MHz, ^{13}C: 50 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (= 0 ppm); J values in Hz; some compounds show the presence of rotamers which are indicated. MS: *VG Tribrid* (EI) or *Hitachi Perkin-Elmer RHU-6M* (FAB, in a 3-nitrobenzyl-alcohol matrix) spectrometer; in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Benzoylation of β-Amino-Acid Derivatives* **14c** *and* epi-**14b**: *General Procedure 1* (*GP 1*). The Boc-protected amino-acid derivative was Boc-deprotected according to *GP 5a*. The resulting TFA salt was dissolved at 0° in CH_2Cl_2 (0.2M) and treated with Et_3N (5 equiv.), BzCl (1.2 equiv.), and a catal. amount of DMAP. The mixture was stirred for 16 h, diluted with CH_2Cl_2, and washed with sat. aq. NH_4Cl and NaCl solns. The org. phase was dried ($MgSO_4$) and evaporated. FC yielded the pure product.

3. *Amidomethylation of the Acyl-oxazolidinones* **21**: *General Procedure 2* (*GP 2*). The oxazolidinone **21** was dissolved in CH_2Cl_2 (0.25M) and cooled (ice-bath). $TiCl_4$ (1 equiv.) was slowly added (internal temp. < 5°) and the resulting yellow soln. (or suspension) stirred for 10 min. Then, Et_3N (1 equiv.) was added (internal temp. < 5°) and the resulting dark red soln. stirred for 45 min at 0°. *N*-(Chloromethyl)benzamide (1.2 equiv.) was added and the mixture stirred for another 60 min at 0°. The mixture was treated with sat. NH_4Cl soln., the org. layer washed with 1M HCl soln., and the HCl phase extracted with CH_2Cl_2. The combined org. phases were dried ($MgSO_4$) and evaporated. The resulting crude product was purified by FC or recrystallization.

4. *Cleavage of the Chiral Auxiliary: General Procedure 3* (*GP 3*). The acyl-oxazolidinone **22** was dissolved in THF/H_2O 4:1 (0.2M) and cooled (ice-bath). H_2O_2 (30% aq. soln., 4 equiv.) and $LiOH \cdot H_2O$ (1.6 equiv.) were added, and the soln. was stirred for 60 min at 0°. The mixture was treated with sat. $NaHSO_3$ soln. (4 equiv.) and washed with CH_2Cl_2. The aq. phase was acidified (pH 1–2) with 6M HCl and extracted with AcOEt (3 ×). The combined AcOEt phases were dried ($MgSO_4$) and evaporated. The resulting crude product was purified by recrystallization. The chiral auxiliary was recovered from the CH_2Cl_2 phase.

5. *Cleavage of the Benzoyl Group: General Procedure* 4 (*GP 4*). The Bz-protected amino acid **23** was dissolved in conc. HCl/AcOH/H_2O 2:1:1 (0.25M) and refluxed for 2 d (bath-temp. 110°). The mixture was diluted with H_2O and washed with Et_2O. The aq. phase was evaporated. The crude amino-acid hydrochloride was purified by ion-exchange chromatography (*Dowex 50-8*, strongly acidic, elution with 1% aq. ammonia soln.) and recrystallization of the amino acid.

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

GP 5b: Similarly to the reported procedure [4][5], the Boc-protected amino acid was dissolved in TFA (0.25M) at 0°. After stirring at r.t. for 2 h, concentration under reduced pressure and drying of the residue under h.v. yielded the crude TFA salt, which was used without further purification.

GP 5c: The Boc-protected amino acid or peptide was dissolved (0.25M) in sat. HCl/dioxane and stirred for 1–2 h at r.t. Concentration under reduced pressure and drying under h.v. yielded the crude HCl salt, which was used without further purification.

7. *Benzyl-Ester Deprotection: General Procedure 6 (GP 6)*. The benzyl ester was dissolved in the appropriate solvent (0.1M) and a catal. amount of 10% Pd/C was added. The apparatus was evacuated and flushed three times with H_2, and the mixture was stirred under H_2 for 18 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded the crude carboxylic acid, which was identified by NMR and used without further purification.

8. *Transesterification of β-Amino-Acid Derivatives* **14**: *General Procedure 7 (GP 7)*. The appropriate methyl ester was dissolved in BnOH (0.5M). A soln. of $Ti(OBn)_4$ in BnOH (0.7–4 equiv., 0.58M) and molecular sieves (4 Å) was added. This mixture was heated at 95° for 40–60 h (NMR control). After filtration over *Celite* and dilution with Et_2O, the org. phase was washed thoroughly with aq. KF (pH 1), sat. aq. $NaHCO_3$, and NaCl solns., and then dried ($MgSO_4$). The solvent was removed *in vacuo* and the BnOH was then removed by bulb-to-bulb distillation (100°/0.1 Torr). The resulting crude product was purified by FC.

9. *Peptide Coupling with EDC: General Procedures 8 (GP 8)*. *GP 8a*: The appropriate TFA salt was dissolved in $CHCl_3$ (0.5M) and cooled to 0°. This was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.25M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and then stirred for 18 h. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1M HCl, and sat. aq. $NaHCO_3$ and NaCl solns. The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC yielded the pure peptide.

GP 8b: The appropriate TFA salt was dissolved in $CHCl_3$ and washed with 1N NaOH (3×). The H_2O phase was extracted with $CHCl_3$. The combined org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. The resulting amino compound was dissolved in $CHCl_3$ (0.4M) and cooled to 0°. This was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.4M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and then stirred for 18 h. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1M HCl, sat. aq. $NaHCO_3$ and NaCl solns. The org. phase was dried ($MgSO_4$) and then concentrated *in vacuo*. FC yielded the pure peptide.

GP 8c: The appropriate HCl, TFA, or TsOH salt (1.0 equiv.) was dissolved in THF or CH_2Cl_2. The Boc-protected fragment (1.0 equiv.) and HOBt (1.2 equiv.) were added, and the mixture was cooled to 0° (ice-bath) and successively treated with NMM (2.8 equiv.) and EDC (1.0 equiv.). The soln. was stirred for 1 h at 0° then at r.t. for 18 h. The mixture was diluted with AcOEt and washed with 0.5M HCl (3×), sat. K_2CO_3 (3×), and sat. NaCl solns.; the org. phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude peptide was purified by FC or recrystallization.

Boc-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-OBn (**1a**). Compound *ent*-**29a** (107 mg, 0.20 mmol) was deprotected according to *GP 5c*, dissolved in THF (8 ml), and treated with *ent*-**29b** (89 mg, 0.20 mmol), HOBt (34 mg, 0.25 mmol), NMM (0.07 ml, 0.60 mmol), and EDC (42 mg, 0.22 mmol) according to *GP 8c*. FC (CH_2Cl_2/MeOH 19:1) yielded **1a** (119 mg, 69%). White solid. $[\alpha]_D^{r.t.} = -110.2$ ($c = 0.95$, $CHCl_3$). R_f 0.20 (CH_2Cl_2/MeOH 19:1). IR ($CHCl_3$): 3445*m*, 3307*m*, 3008*m*, 2965*s*, 2873*m*, 1702*m*, 1654*s*, 1522*s*, 1469*m*, 1368*m*, 1170*s*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.88–0.93 (*m*, 6 Me); 0.96 (*d*, $J = 6.6$, 2 Me); 1.03 (*d*, $J = 7.0$, Me); 1.16 (*d*, $J = 7.1$, Me); 1.24–1.29 (*m*, 2 CH); 1.43 (*s*, *t*-Bu); 1.52–1.63 (*m*, 2 CH_2); 1.80–1.85 (*m*, CH); 1.89–1.92 (*m*, CH); 1.99–2.05 (*m*, CHCO); 2.06–2.11 (*m*, CHCO); 2.42–2.52 (*m*, 3 CHCO); 2.71–2.76 (*m*, CHCO); 3.05–3.75 (*m*, 6 CH_2N); 5.10 (*d*, $J = 12.3$, 1 H, $PhCH_2$); 5.16 (*d*, $J = 12.2$, 1 H, $PhCH_2$); 5.54 (*t*, $J = 5.9$, N*H*Boc); 6.80 (br., NH); 7.02 (br., NH); 7.09 (br., NH); 7.15 (br., NH); 7.31–7.37 (*m*, 5 arom. H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 15.4, 15.5, 20.3, 20.4, 21.0, 21.1, 22.3, 22.6, 23.1 (Me); 25.9, 26.0, 28.4, 28.4 (CH); 28.5 (Me); 38.8, 38.8, 39.8 (CH_2); 40.8 (CH); 41.0, 41.2 (CH_2); 42.0, 42.3, 42.6, 42.8, 44.3, 45.3, 54.8, 55.1 (CH); 66.7 (CH_2); 79.0 (C); 128.2, 128.3, 128.6 (CH); 135.9, 156.3, 174.6, 174.6, 174.9, 175.2, 175.3, 175 (C). FAB-MS: 860 (18, $[M + 1]^+$), 859 (30, M^+), 764 (10), 761 (20), 760 (59), 759 (100), 91 (21).

H-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-OH · CF₃COOH (**1c**). Compound **1a** (98 mg, 0.11 mmol) was dissolved in CH_2Cl_2/TFA 1:1 (1 ml) and stirred for 4.5 h at r.t. The solvent was evaporated, the residue dried under h.v. and dissolved in MeOH (5 ml). The soln. was treated with 10% Pd/C (15 mg) in MeOH according to *GP 6*. FC (CH_2Cl_2/MeOH 15:1) and addition of TFA yielded **1c** (83 mg, 96%). Colorless glass. CD (0.2 mM in MeOH): $+ 3.3 \cdot 10^4$ (216), $-7.9 \cdot 10^4$ (198). ^{1}H-NMR (500 MHz, CD_3OD): 0.90–0.99 (*m*, 8 Me); 1.10 (*d*, $J = 6.7$, Me); 1.12 (*d*, $J = 6.7$, Me); 1.15–1.21 (*m*, CH); 1.24–1.32 (*m*, CH); 1.45–1.57 (*m*, 1 H, CH_2); 1.60–1.68 (*m*, 1 H, CH_2); 1.76–1.85 (*m*, CH); 1.85–1.92 (*m*, CH); 2.25–2.29 (*m*, CHCO); 2.47–2.56 (*m*, CHCO); 2.57–2.67 (*m*, CH_2CO); 2.69–2.75 (*m*, CHCO); 2.76–2.82 (*m*, CHCO); 3.09–3.22 (*m*, CH_2N); 3.25–3.32 (*m*, 3 CH_2N); 3.34–3.41 (*m*, CH_2N); 3.51 (*dd*, $J = 13.2, 10.7$, 1 H, CH_2N); 3.59 (*dd*, $J = 13.3, 10.1$, 1 H, CH_2N). ^{13}C-NMR (125 MHz, CD_3OD): 16.2, 16.6, 20.1, 20.7, 20.9, 21.1, 22.5, 22.8, 23.3, 23.7 (Me); 27.1, 27.4, 30.4, 30.8 (CH); 40.6, 40.6, 40.7, 41.1 (CH_2); 41.7, 41.9 (CH); 42.6, 42.8, 43.4, 43.4 (CH_2); 44.9, 45.6, 51.7, 54.0 (CH); 174.3, 175.7, 176.8, 177.2, 177.6, 178.6 (C). FAB-MS: 1383 (2, $[2M + Na]^+$), 692 (80), 691 (100, $[M + Na]^+$), 669 (90, $[M + 1]^+$).

Boc-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-(2R,3R)-β²,³-HAla(α-Me)-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-OBn (**2a**). Compound **33a** (148 mg, 0.23 mmol) was deprotected according to *GP 5c*, the HCl salt dissolved in THF (10 ml) and treated with *ent*-**29b** (111 mg, 0.25 mmol), HOBt (41 mg, 0.30 mmol), NMM (0.08 ml, 0.70 mmol), and EDC (50 mg, 0.26 mmol) according to *GP 8c*. FC (CH_2Cl_2/MeOH 12:1) yielded **2a** (106 mg, 48%). White solid. M.p. 200° (dec.). ^{1}H-NMR (500 MHz, DMSO): 0.75–0.96 (*m*, 12 Me); 1.00–1.35 (*m*, 8 CH); 1.35 (*s*, *t*-Bu); 1.40–1.50 (*m*, 2 CH); 1.65–1.73 (*m*, CHCO); 2.06–2.12 (*m*, CHCO); 2.33–2.52 (*m*, 3 CHCO); 2.61–2.68 (*m*, CHCO); 2.94–3.15 (*m*, 9 H, CHN, CHCO); 3.18–3.40 (*m*, 3 CHN); 3.82–3.90 (*m*, CHN); 5.07 (*s*, CH_2O); 6.39 (br., NH); 7.28–7.39 (*m*, 5 arom. H); 7.48–7.82 (br., 5 NH); 7.99 (br., NH). ^{13}C-NMR (125 MHz, DMSO): 14.3, 15.6, 15.7, 18.1, 20.0, 20.1, 20.2, 21.7, 21.9, 22.7, 23.1, 25.4, 25.5, 27.9, 28.1, 43.1, 43.2, 44.3, 46.6, 51.6, 51.7, 65.5, 77.4, 127.8, 127.9, 128.3, 136.0, 155.3, 172.4, 173.0, 173.1, 173.9, 174.2, 174.4. FAB-MS: 997 (19, $[M + K]^+$), 981 (100, $[M + Na]^+$), 907 (28), 881 (35), 859 (52).

H-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-(2R,3R)-β²,³-HAla(α-Me)-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-OH · CF₃COOH (**2c**). Compound **2a** (80 mg, 0.08 mmol) was dissolved in CH_2Cl_2/TFA 1:1 (2 ml) and stirred for 2 h at r.t. The solvent was evaporated, the residue dried under h.v., and dissolved in MeOH (5 ml), and treated with 10% Pd/C (15 mg) in MeOH according to *GP 6*. FC (CH_2Cl_2/MeOH 10:1) and addition of TFA yielded **2c** (58 mg, 79%). Glass. CD (0.2 mM in MeOH): $+ 1.1 \cdot 10^5$ (216), $-0.9 \cdot 10^5$ (194). IR ($CHCl_3$): 3272*m*, 2964*m*, 1643*s*, 1555*m*, 1454*m*, 1178*m*, 1144*m*, ^{1}H- (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): data in *Tables 4* and *5*. FAB-MS: 807 (80, $[M + K]^+$), 791 (42, $[M + Na]^+$), 769 (100, $[M + 1]^+$).

Boc-(S)-β²-HVal-(S)-β²-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-OBn (**3a**). Treatment of a soln. of **29a** (0.62 g, 1.17 mmol) in MeOH (25 ml) with Pd/C (70 mg) according to *GP 6* yielded **29b** (0.55 g). Compound **32a** (0.46 g, 0.87 mmol) was deprotected according to *GP 5a*, the resulting TFA salt dissolved in CH_2Cl_2 (2 ml) and treated with **29b** (0.39 g, 0.88 mmol), HOBt (0.15 g, 0.95 mmol), NMM (0.27 ml, 2.56 mmol), and EDC (0.17 g, 0.88 mmol) according to *GP 8c*. FC ($CHCl_3$/Et_2O/MeOH 73:23:4 → $CHCl_3$/MeOH 96:4) yielded **3a** (0.28 g, 37%). Glass. M.p. 221–222°. R_f 0.12 ($CHCl_3$/Et_2O/MeOH 73:23:4). $[\alpha]_D^{r.t.} = + 89.0$ (*c* = 0.57, $CHCl_3$). CD (0.2 mM in MeOH): $+ 1.2 \cdot 10^5$ (208). IR ($CHCl_3$): 3440*m*, 3300*m*, 3070*w*, 3000*m*, 2955*m*, 2930*m*, 2870*w*, 1700*m*, 1650*s*, 1540*m*, 1460*w*, 1450*w*, 1440*m*, 1170*m*, 1140*m*, 1120*m*, 1035*w*, 1005*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.98 (*d*, *J* = 6.6, Me); 0.79–0.99 (*m*, 7 Me); 0.95–1.09 (*m*, CH); 1.23 (*d*, *J* = 7.1, Me); 1.19–1.35 (*m*, 2 CH); 1.38–1.45 (*m*, Me); 1.47 (*s*, *t*-Bu); 1.38–1.96 (*m*, 6 CH); 2.01–2.20 (*m*, 2 CH); 2.26–2.57 (*m*, 5 CH); 2.58–2.68 (*m*, CH); 2.72–2.85 (*m*, CH); 2.86–3.02 (*m*, 2 CH); 3.53–3.74 (*m*, 3 CHN); 3.97–4.10 (*m*, CHN); 4.15–4.27 (*m*, CHN); 4.36–4.50 (*m*, CHN); $\nu_A = 5.03$, $\nu_B = 5.13$ (*AB*, $J_{AB} = 12.1$, CH_2O); 5.88 (br., NH); 6.50 (br. NH); 6.91 (br., NH); 7.32–7.43 (5 arom. H); 7.89 (br. 2 NH); 8.24 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.9, 17.4, 19.8, 20.0, 20.1, 20.8, 21.5, 22.0, 23.1, 23.6 (Me); 24.9, 25.9, 28.4 (CH); 28.6 (CH_3); 32.6 (CH); 38.7, 41.2, 41.4, 42.0, 42.8, 42.8, 43.5 (CH_2); 44.5, 45.3, 47.2, 52.6, 55.0 (CH); 67.0 (CH_2); 76.7 (CH); 78.7 (C); 128.4, 128.5, 128.6 (CH); 135.7, 156.6, 171.8, 172.6, 174.4, 175.2 (C). FAB-MS: 881 (12), 859 (17, M^+), 761 (12), 760 (45), 759 (100), 91 (14), 73 (10).

H-(S)-β²-HVal-(S)-β²-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-OH · CF₃COOH (**3c**). Compound **3a** (89 mg, 0.10 mmol) was deprotected according to *GP 5a*, the resulting TFA salt **3b** dissolved in MeOH (2 ml) and treated with Pd/C (10 mg) according to *GP 6*. Prep. RP-HPLC (MeCN/H_2O (0.1% TFA) 65:35) yielded **3c** (26 mg, 32%). CD (0.2 mM in MeOH): $+ 4.7 \cdot 10^4$ (200), $-2.7 \cdot 10^4$ (216). ^{1}H-NMR (400 MHz, CD_3OD): 0.87–1.01 (*m*, 8 Me); 1.06–1.15 (*m*, 2 Me); 1.06–1.21 (*m*, CH); 1.22–1.35 (*m*, CH); 1.39–1.67 (*m*, 4 CH); 1.70–1.92 (*m*, 2 CH); 2.20–2.55 (*m*, 6 CH); 2.64–2.79 (*m*, 2 CH); 2.87–3.00 (*m*, 2 CH); 3.01–3.25 (*m*, 3 CH); 3.38–3.48 (*m*, CH); 3.77–3.90 (*m*, CH); 4.15–4.25 (*m*, CHN); 4.30–4.45 (*m*, 2 CHN); 7.84 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 16.0, 19.0, 19.7, 20.3, 20.8, 21.0, 22.4, 22.5, 23.6, 23.6 (Me); 26.0, 27.1, 30.5, 33.9 (CH); 38.9, 40.7, 40.9, 41.4 (CH_2); 42.1 (CH); 42.6 (CH_2); 43.4 (CH); 43.5 (CH_2); 43.7, 45.3 (CH); 45.4 (CH_2); 46.0, 52.0, 53.5 (CH); 172.0, 172.0, 174.0, 174.9, 176.3, 177.8 (C). FAB-MS: 713 (13), 692 (33), 691 (73), 683 (14), 671 (12), 670 (47), 669 (100).

Boc-(S)-β²-HVal-(S)-β³-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-OBn (**4a**). Treatment of a soln. of **30a** (0.46 g, 0.87 mmol) in MeOH (18 ml) with Pd/C (50 mg) according to *GP 6* yielded **30b** (0.64 g, 96%). Compound **31a** (130 mg, 0.25 mmol) was deprotected according to *GP 5a*, the resulting TFA salt dissolved in CH_2Cl_2 (1 ml) and treated with **30b** (110 mg, 0.25 mmol), HOBt (50 mg, 0.32 mmol), NMM (0.08 ml, 0.70 mmol), and EDC (50 mg, 0.26 mmol) according to *GP 8c*. FC (CH_2Cl_2/MeOH 96:4) yielded **4a** (110 mg, 51%). Glass. M.p. 209–210°. R_f 0.23 (CH_2Cl_2/MeOH 96:4). $[\alpha]_D^{r.t.} = + 141.10$ (*c* = 0.80, $CHCl_3$). CD (0.2 mM in MeOH): $+ 3.1 \cdot 10^5$ (203). IR ($CHCl_3$): 3440*w*, 3300*m*, 3080*w*, 3005*w*, 2975*m*, 2930*m*, 2880*w*, 1705*m*, 1645*s*, 1560*m*, 1520*m*, 1455*w*, 1430*w*, 1390*w*, 1370*w*, 1325*w*, 1305*w*, 1285*m*, 1175*m*, 1120*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.82–0.96 (*m*, 7 Me), 0.99 (*d*, *J* = 6.6, Me); 1.06 (*d*, *J* = 6.8, Me); 1.24 (*d*, *J* = 6.8, Me); 1.21–1.32 (*m*, CH); 1.48 (*s*, *t*-Bu); 1.42–1.57 (*m*, 2 CH); 1.58–1.69 (*m*, CH); 1.70–2.15 (*m*, 7 CH); 2.18–2.39 (*m*, 2 CH); 2.45–2.80

(*m*, 6 CH); 2.91–3.05 (*m*, CH); 3.55–3.75 (*m*, 3 CHCO); 4.28–4.47 (*m*, 2 CHN); 4.59–4.73 (*m*, CHN); ν_A = 5.03, ν_B = 5.16 (*AB*, J_{AB} = 12.2, CH_2O); 5.52 (br., NHBoc); 6.78 (br., NH); 7.31–7.44 (5 arom. H); 7.55 (br., NH); 8.25 (br., NH); 8.43 (br., NH); 8.60 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.5, 17.2, 19.9, 20.2, 20.6, 21.4, 21.9, 23.1, 23.7 (Me); 24.8, 26.0 (CH); 28.6 (Me); 32.6 (CH); 38.2 (CH_2); 40.0 (CH); 41.4, 41.8, 42.4, 42.7, 43.6, 43.9 (CH_2); 44.1, 44.4 (CH); 45.7 (CH_2); 45.7, 53.4, 54.9 (CH); 67.3 (CH_2); 78.6 (C); 128.4, 128.5, 128.6 (CH); 135.6, 156.7, 171.5, 172.7, 173.6, 174.0, 175.0, 175.3 (C). FAB-MS: 1739 (12), 884 (24), 883 (63), 882 (100, $[M + Na]^+$), 861 (13), 860 (25, $[M + 1]^+$), 782 (13), 762 (14), 761 (45), 760 (83). Anal. calc. for $C_{46}H_{78}N_6O_9$ (859.16): C 64.31, H 9.15, N 9.78; found: C 64.49, H 9.12, N 9.57.

H-(S)-β²-HVal-(S)-β²-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-OH · HCl (**4d**). Compound **4a** (100 mg, 0.12 mmol) was deprotected according to *GP 5a*, the resulting TFA salt **4b** dissolved in MeOH (2.5 ml) and treated with Pd/C (10 mg) according to *GP 6*. Prep. RP-HPLC (MeCN/H_2O (0.1% TFA) 60:40) yielded **4d** (45 mg, 47%) as TFA salt. Repeated dissolving in 0.1M HCl gave the HCl salt of **4d**. CD (0.2 mM **4c** in MeOH): $+ 1.6 \cdot 10^5$ (202). ^{1}H-NMR (400 MHz, CD_3OD): 0.75–1.12 (*m*, 9 Me); 1.25 (*d*, *J* = 7.6, Me); 1.28–1.39 (*m*, CH); 1.43–1.91 (*m*, 6 CH); 2.09–2.25 (*m*, 2 CH); 2.36–2.63 (*m*, 6 CH); 2.64–2.75 (*m*, CH); 2.85–2.98 (*m*, CH); 3.02–3.21 (*m*, 3 CH); 3.27–3.45 (*m*, 3 CH); 4.19–4.30 (*m*, CHN); 4.31–4.44 (*m*, CHN); 4.46–4.57 (*m*, CHN); 8.27 (br., NH); 8.33 (br., NH); 8.42 (br., NH); 8.55 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 15.6, 17.9, 20.1, 20.5, 20.6, 21.2, 22.1, 22.1, 23.7, 24.0 (Me); 26.0, 27.2, 30.3, 33.8 (CH); 40.1, 41.3, 41.5, 41.5 (CH_2); 41.7 (CH); 43.4, 44.0 (CH_2); 44.9 (CH); 45.0, 45.0 (CH_2); 45.6, 46.2, 52.5, 54.3 (CH); 173.5, 174.0, 174.4, 175.6, 176.5, 176.8 (C). FAB-MS: 708 (15), 707 (15), 693 (25), 692 (54), 691 (56), 672 (20), 671 (63), 670 (99), 669 (100).

Boc-(S)-β²-HVal-(S)-β³-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-(S)-β²-HVal-(S)-β³-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-OBn (**5a**). Treatment of a soln. of **4a** (79.2 mg, 0.09 mmol) in MeOH (2 ml) with Pd/C (10 mg) according to *GP 6* yielded **4b**. Compound **4a** (81.9 mg, 0.10 mmol) was deprotected according to *GP 5a*, the resulting TFA salt dissolved in CH_2Cl_2 (1 ml) and treated with **4b**, HOBt (16 mg, 0.10 mmol), NMM (0.03 ml, 0.27 mmol), and EDC (19 mg, 0.10 mmol) according to *GP 8c*. FC (CH_2Cl_2/Et_2O/MeOH 48:48:4) yielded **5a** (79 mg, 57%). Colorless needles. M.p. 220–221°. R_f 0.66 (CH_2Cl_2/Et_2O/MeOH 48:48:4). $[\alpha]_D^{r.t.}$ = + 216.38 (*c* = 0.81, $CHCl_3$). CD (0.2 mM in MeOH): $+ 6.0 \cdot 10^5$ (204). IR ($CHCl_3$): 3700*w*, 3430*w*, 3280*m*, 3100*w*, 2970*m*, 2935*m*, 2880*w*, 1705*w*, 1640*s*, 1565*m*, 1460*w*, 1435*m*, 1395*w*, 1375*w*, 1355*w*, 1310*w*, 1265*w*, 1175*m*, 1120*w*, 1095*w*, 1035*w*, 1110*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.78–1.10 (*m*, 18 Me); 1.20–1.32 (*m*, 2 Me); 1.48 (*s*, *t*-Bu); 1.20–2.42 (*m*, 34 CH); 2.45–2.80 (*m*, 13 CH); 2.94–3.04 (*m*, CH); 3.55–3.77 (*m*, 5 CH); 3.90–3.99 (*m*, 2 CH); 4.03–4.15 (*m*, CH); 4.28–4.75 (*m*, 6 CH); ν_A = 5.04, ν_B = 5.17 (*AB*, J_{AB} = 12.1, CH_2O); 6.76 (br., NH); 7.31–7.47 (*m*, 5 arom. H); 7.51–7.59 (br., NH); 8.34–8.51 (*m*, 3 NH); 8.65–8.97 (*m*, 5 NH); 9.03 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.3, 14.5, 17.0, 17.0, 19.9, 20.0, 20.1, 20.2, 20.6, 20.8, 21.3, 21.5, 21.8, 21.9, 23.1, 23.3, 23.6, 23.8 (Me); 24.4 (CH_2); 24.8, 24.8 (CH); 25.6 (CH_2); 26.0, 26.1, 27.6 (CH); 28.5, 28.6 (Me); 29.7 (CH_2); 32.4, 32.5 (CH); 33.9 (CH_2); 38.3, 39.7 (CH); 39.9, 40.1, 41.4, 41.8, 42.3, 42.4, 42.8 (CH_2); 43.5 (CH); 43.6 (CH_2); 43.9, 44.1, 44.3 (CH); 44.4, 45.3, 45.6 (CH_2); 45.7 (CH); 46.0 (CH_2); 46.5, 53.3, 53.4, 53.5, 54.9 (CH); 64.3, 67.3 (CH_2); 78.5, 128.4, 128.5, 128.6 (CH); 135.6, 156.7, 171.6, 172.3, 172.7, 172.8, 173.2, 173.6, 173.4, 174.3, 174.7, 175.1, 175.2, 175.4 (C). FAB-MS: 1624 (10), 1535 (26), 1534 (509), 1533 (48), 1532 (17, $[M + Na]^+$), 1512 (12), 1511 (14), 1414 (24), 1413 (64), 1412 (100), 1411 (18), 1411 (80), 1410 (46), 182 (10).

H-(S)-β²-HVal-(S)-β³-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-(S)-β²-HVal-(S)-β³-HAla-(S)-β²-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-OH · CF₃COOH (**5c**). Compound **5a** (79 mg, 0.05 mmol) was deprotected according to *GP 5a*, the resulting TFA salt **5b** dissolved in MeOH (2 ml) and treated with Pd/C (10 mg) according to *GP 6* to yield **5c** (56 mg, 74%). Glass. For anal. purposes **5c** was further purified by prep. RP-HPLC (MeCN/H_2O (0.1% TFA) 50:50). CD (0.2 mM in MeOH): $+ 4.9 \cdot 10^4$ (203), $- 2.6 \cdot 10^4$ (215). ^{1}H-NMR (500 MHz, CD_3OD): 0.75–1.04 (*m*, 12 Me); 1.05–1.16 (*m*, 2 Me); 1.20–1.49 (*m*, 6 Me); 1.20–1.90 (*m*, 18 CH); 1.92–2.85 (*m*, 13 CH); 2.90–3.00 (*m*, CH); 3.02–3.26 (*m*, 6 CH); 3.26–3.42 (*m*, 4 CH); 3.42–3.70 (*m*, 6 CH); 4.17–4.62 (*m*, 4 CHN); 6.77–6.81 (br., NH); 7.08–7.12 (br., NH); 7.25–7.35 (*m*, 3 NH); 7.35–7.40 (br., NH); 7.51 (br., NH); 8.40–8.52 (br., NH); 8.57 (br., NH); 8.69 (br., NH); 8.72–8.79 (br., NH). FAB-MS: 1365 (14), 1363 (21), 1362 (27), 1361 (25), 1360 (16), 1359 (11), 1340 (11), 1339 (14), 1338 (15), 1337 (16), 1328 (20), 1326 (50), 1325 (88), 1324 (98), 1323 (100), 1322 (61), 1321 (27).

Boc-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-(R)-β³-HVal-(S)-β²-HAla-(S)-β³-HLeu-OBn (**6a**). Compound **31a** (150 mg, 0.28 mmol) was Boc-deprotected according to *GP 5a*. The resulting crude TFA salt was coupled with **31b** (0.125 mg, 0.28 mmol) according to *GP 8a*. After the coupling was completed, the solvent was evaporated, and the white precipitate was washed successively with MeOH and MeOH/H_2O 1:1 to yield **6a** (188 mg, 78%). White powder. M.p. 231–233°. $[\alpha]_D^{r.t.}$ = + 34.9 (*c* = 0.65, CF_3CH_2OH). CD (0.2 mM in MeOH): $+ 2.05 \cdot 10^5$ (201). IR (KBr): 3303*m*, 3060*w*, 2960*s*, 2873*m*, 2477*m*, 1734*s*, 1684*s*, 1636*s*, 1540*s*, 1457*s*, 1390*m*, 1367*m*, 1310*m*, 1251*m*, 1174*s*, 1125*m*, 1100*m*, 1050*m*, 1025*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.72–0.87

(*m*, 8 Me); 0.87–1.00 (*m*, 2 Me); 1.09–1.22 (*m*, 2 CH); 1.29–1.42 (*m*, 2 CH); 1.36 (*s*, *t*-Bu); 1.47–1.80 (*m*, 4 CH); 2.00–2.45 (*m*, 9 CH); 2.52–2.62 (*m*, CH); 2.84–3.22 (*m*, 4 CHN); 3.62–3.72 (*m*, CHN); 3.90–4.03 (*m*, 2 CHN); 4.07–4.23 (*m*, CHN); $\nu_A = 5.05$, $\nu_B = 5.11$ (*AB*, $J_{AB} = 12.4$, CH_2O); 6.55–6.64 (br., NH); 7.30–7.41 (5 arom. H); 7.60–7.88 (*m*, 5 NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 15.4, 17.7, 17.8, 18.1, 19.0, 21.3, 21.5, 23.1, 23.3 (Me); 24.2 (CH); 28.1 (Me); 31.0, 31.3, 31.8 (CH); 38.1, 38.3, 40.4, 41.7, 42.1, 42.8, 43.1 (CH_2); 43.6, 44.3, 51.1, 52.5, 52.9 (CH); 65.4 (CH_2); 77.3 (C), 128.0, 128.3 (CH); 136.0, 155.3, 169.9, 170.2, 170.5, 170.7, 173.1, 173.5 (C). FAB-MS: 882 (113), 881 (28), 860 (12), 859 (23), 761 (12), 760 (50), 759 (100), 646 (12).

Boc(2S,3S)-β^{2,3}-HVal(α-Me)-(2S,3S)-β^{2,3}-HAla(α-Me)-(2S,3S)-β^{2,3}-HLeu(α-Me)-(2S,3S)-β^{2,3}-HVal-(α-Me)-(2S,3S)-β^{2,3}-HAla(α-Me)-(2S,3S)-β^{2,3}-HLeu(α-Me)-OBn (**7a**). Compound **34a** (86.7 mg, 0.15 mmol) was Boc-deprotected according to *GP 5a*. The resulting crude TFA salt was coupled with **34b** (73 mg, 0.15 mmol) according to *GP 8a*. FC (CH_2Cl_2/MeOH 25:1 to 10:1) yielded **7a** (77.7 mg, 55%). Colorless glass which gave a voluminous colorless powder after lyophilization from dioxane. M.p. 222–223.5°. R_f 0.21 ($CHCl_3$/MeOH 10:1). $[\alpha]_D^{r.t.} = -34.5$ (*c* = 1.0, $CHCl_3$). CD (0.2 mM in MeOH): $+2.87 \cdot 10^4$ (199), $-1.13 \cdot 10^4$ (220). IR ($CHCl_3$): 3386*w*, 3007*m*, 2968*m*, 2934*m*, 2875*w*, 1702*m*, 1649*s*, 1494*s*, 1456*m*, 1389*m*, 1368*m*, 1290*m*, 1174*m*, 1047*w*, 977*w*, 648*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–1.00 (*m*, 8 Me); 1.14–1.25 (*m*, 8 Me); 1.27–1.42 (*m*, *t*-Bu, 3 CH); 1.50–1.70 (*m*, 5 CH); 2.24–2.35 (*m*, 2 CHCO); 2.42 (*dq*, *J* = 7.0, 3.1, CHCO); 2.51–2.62 (*m*, 2 CHCO); 2.73 (*dq*, *J* = 7.2, 3.5, CHCO); 3.27 (*dt*, *J* = 9.5, 3.6, CHN); 3.59 (*dt*, *J* = 9.4, 2.9, CHN); 3.96–4.06 (*m*, 2 CHN); 4.10–4.17 (*m*, 2 CHN); 5.11 (*d*, *J* = 12.2, 1 H, PhCH_2); 5.17 (*d*, *J* = 12.2, 1 H, PhCH_2); 6.12 (*d*, *J* = 9.9, NH); 6.39 (*d*, *J* = 9.8, NH); 7.32–7.40 (*m*, 5 arom. H); 7.56 (*d*, *J* = 8.9, 2 NH); 7.61–7.64 (*m*, 2 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.47, 16.69, 16.71, 16.96, 16.99, 19.77, 19.93, 20.04, 20.09, 20.19, 20.32, 22.14, 22.49, 22.57, 22.79, 25.03 (Me); 25.05 (CH); 28.48 (Me); 29.71, 32.39, 32.45, 40.13, 41.24, 42.20, 42.92 (CH); 43.23; 44.00 (CH_2); 44.49, 44.54, 47.42, 47.52, 49.29, 49.78, 57.74, 59.35, 66.58 (CH_2); 78.16 (C); 128.21, 128.57, 128.72 (CH); 135.53, 156.82, 175.32, 175.46, 175.59, 175.90 (C). FAB-MS: 981 (66, $[M + K]^+$), 966 (100, $[M + Na]^+$), 875 (6), 844 (63). Anal. calc. for $C_{52}H_{90}N_6O_9$ (943.32): C 66.21, H 9.62, N 8.91; found: C 65.97, H 9.90, N 8.62.

H-(2S,3S)-β^{2,3}-HVal(α-Me)-(2S,3S)-β^{2,3}-HAla(α-Me)-(2S,3S)-β^{2,3}-HLeu(α-Me)-(2S,3S)-β^{2,3}-HVal-(α-Me)-(2S,3S)-β^{2,3}-HAla(α-Me)-(2S,3S)-β^{2,3}-HLeu(α-Me)-OH · CF$_3$COOH (**7c**). Compound **7a** (46.5 mg, 0.049 mmol) was debenzylated in MeOH according to *GP 6* affording the corresponding acid (45 mg, 100%) which was Boc-deprotected according to *GP 5a* to give a colorless glass, **7c**. The peptide was purified by prep. RP-HPLC (MeCN (0.08% TFA)/H_2O (0.1% TFA) 1:1). The product was obtained as a voluminous colorless powder after lyophylization from dioxane (31.4 mg, 72% after HPLC). M.p. 240° (dec.). R_f 0.38 (CH_2Cl_2/MeOH 13:1, 1% AcOH). $[\alpha]_D^{r.t.} = +14.2$ (*c* = 0.6, MeOH). CD (0.2 mM in MeOH): $+1.52 \cdot 10^5$ (198), $-5.16 \cdot 10^4$ (217). IR (KBr): 3286*s*, 3086*m*, 2969*s*, 2936*s*, 2879*m*, 1717*s*, 1653*s*, 1557*s*, 1545*s*, 1458*m*, 1384*m*, 1368*m*, 1261*w*, 1241*m*, 1194*s*, 1138*s*, 974*w*, 947*w*, 931*w*, 913*w*, 847*w*, 831*w*, 800*w*, 720*m*. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see *Tables 7* and *8*. FAB-MS: 1546 (26, $[2M + K]^+$), 1508 (6, $[2M + 1]^+$), 792 (94, $[M + K]^+$), 777 (40, $[M + Na]^+$), 755 (100, $[M + 1]^+$).

*Benzyl (S)-3-{[(*tert*-Butoxy)carbonyl]amino}-5-methylhexanoate* (Boc-(*S*)-β^3-HLeu-OBn; **9c**). (*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-5-methylhexan-2-one (7.85 g, 30.8 mmol) was dissolved in THF (106 ml) and BnOH (19 ml) under Ar and cooled to −25° (bath temp.) under exclusion of light. A soln. of $PhCO_2Ag$ (0.77 g, 3.38 mmol) in Et_3N (12.5 ml) was added and the mixture stirred for 3 h while warming to r.t. The solvent was evaporated and the residue dissolved in AcOEt and washed with sat. $NaHCO_3$, NH_4Cl, und NaCl solns. The org. phase was dried ($MgSO_4$) and evaporated. Excess of BnOH was removed by bulb-to-bulb distillation (52°/0.075 Torr). FC (Et_2O/pentane 3:1 → Et_2O/pentane 1:1) yielded **9c** (8.30 g, 80%). White solid. M.p. 42–43.5°. R_f = 0.65 (AcOEt/pentane 1:5). $[\alpha]_D^{r.t.} = -31.5$ (*c* = 1.31, $CHCl_3$). IR ($CHCl_3$): 3425*m*, 3000*m*, 2950*s*, 2925*m*, 1720*s*, 1705*s*, 1500*s*, 1450*m*, 1390*m*, 1360*s*, 1160*s*, 1110*m*, 1020*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, *J* = 6.6, Me_2C); 1.23–1.30 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.34–1.50 (*m*, 1 H, CH_2); 1.58–1.68 (*m*, 1 H, CH_2); 2.50–2.69 (*m*, CH_2CO); 3.94–4.05 (*m*, CHN); 4.85 (br., NH); $\nu_A = 5.10$, $\nu_B = 5.14$ (*AB*, $J_{AB} = 12.3$, CH_2O); 7.29–7.38 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.1, 22.8 (Me); 24.9 (CH); 28.4 (Me); 39.6, 43.6 (CH_2); 45.8 (CH); 66.3 (CH_2); 79.1 (C); 128.2, 128.5 (CH); 135.8, 155.2, 171.5 (C). EI-MS: 336 (0.5, $[M + 1]^+$), 279 (11), 278 (12), 222 (29), 178 (54), 144 (12), 107 (17), 92 (10), 91 (100), 86 (15), 57 (17). Anal. calc. for $C_{19}H_{29}NO_4$ (335.44): C 68.03, H 8.71, N 4.18; found: C 67.85, H 8.64, N 4.22.

*Methyl (R)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-methylpentanoate* (Boc-(*R*)-β^3-HVal-OMe; **10b**). According to [14], a soln. of (*S*)-3-{[(*tert*-butoxy)carbonyl]amino}-1-diazo-4-methylpentan-2-one (8.74 g, 36.2 mmol) in MeOH (145 ml) at −25° under Ar with the exclusion of light was treated with a soln. of $PhCO_2Ag$ (0.91 g, 4.0 mmol) in Et_3N (14.6 ml, 0.105 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 and filtered through *Celite*. After washing with aq. sat. $NaHCO_3$, NH_4Cl, and NaCl solns., the org. phase was dried ($MgSO_4$) and evaporated. FC (AcOEt/pentane 1:7) yielded **10b**

(7.81 g, 88%). Colorless oil which solidified upon refrigeration at −4°. R_f 0.35 (AcOEt/pentane 1:7). $[\alpha]_D^{r.t.} = -28.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3440*m*, 3008*m*, 2972*m*, 2931*m*, 2873*w*, 1731*s*, 1709*s*, 1503*s*, 1439*m*, 1392*m*, 1368*m*, 1168*s*, 1110*m*, 1048*w*, 1019*w*, 858*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, $J = 6.8$, 2 Me); 1.43 (*s*, *t*-Bu); 1.71–1.84 (*m*, 2 Me); 2.45–2.54 (*m*, CH_2CO); 3.68 (*s*, MeO); 3.72–3.79 (*m*, CHN); 4.86 (br. *d*, $J = 8.4$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.5, 19.3, 28.4 (Me); 31.8 (CH); 37.2 (CH_2); 51.7 (Me); 53.0 (CH); 79.1, 155.6, 172.4 (C). EI-MS: 246 (< 1, $[M + 1]^+$), 202 (20), 190 (12), 172 (9), 158 (16), 146 (55), 130 (15), 116 (13), 102 (100), 74 (3), 57 (9). Anal. calc. for $C_{12}H_{23}NO_4$ (245.32): C 58.75, H 9.45, N 5.71; found: C 58.72, H 9.37, N 5.82.

(R)-3-Amino-2-methylpropanoic Acid (H-(*R*)-β^2-HAla-OH; (*R*)-**11a**). Treatment of (*R*)-**23a** (1.04 g, 5 mmol) according to *GP 4* gave, after recrystallization from MeOH, (*R*)-**11a** (385 mg, 75%). Spectoscopic data: in agreement with [41].

(R)-2-(Aminomethyl)-3-methylbutanoic Acid (H-(*R*)-β^2-HVal-OH; (*R*)-**11b**). Treatment of (*R*)-**23b** (6.60 g, 28 mmol) according to *GP 4* gave (*R*)-11b (2.76 g, 75%) which was used without further purification.

(R)-2-(Aminomethyl)-4-methylpentanoic Acid (H-(*R*)-β^2-HLeu-OH; (*R*)-**11c**). Treatment of (*R*)-**23c** (1.25 g, 5 mmol) according to *GP 4* gave, after recrystallization from MeOH, (*R*)-**11c** (469 mg, 65%).

(S)-2-(Aminomethyl)-3-phenylpropanoic Acid (H-(*S*)-β^2-HPhe-OH; (*S*)-**11d**). Treatment of (*S*)-**23d** (2 g, 7.1 mmol) according to *GP 4* gave (*S*)-**11d** (900 mg, 71%). M.p. 219–220°. Spectroscopic data: in agreement with [41]. $[\alpha]_D^{r.t.} = -12.8$ ($c = 1.3$, 1N HCl). [41]: $[\alpha]_D^{r.t.} = -11.0$ ($c = 1.0$, 1N HCl).

Benzyl (R)-2-(Aminomethyl)-4-methylpentanoate Hydrogen p-*Toluenesulfonate* (H-(*R*)-β^2-HLeu-*OBn* · *TsOH*; (*R*)-**12c**). A suspension of (*R*)-**11c** (2.90 g, 20 mmol) and TsOH · H_2O (4.56 g, 24 mmol) in benzene (150 ml) and BnOH (16 ml) was heated to reflux for 9 h, and H_2O formed in the reaction was trapped in a *Dean-Stark* receiver. The clear mixture was cooled to r.t. to yield (*R*)-**12c** (4.70 g, 58%). White needles. Pentane was added to the mother liquor to yield further (*R*)-**12c** (2.34 g, 29%). Total yield of (*R*)-**12c**: 7.04 g (86%). M.p. 135–136.5°. $[\alpha]_D^{r.t.} = -16.6$ ($c = 0.48$, $CHCl_3$). IR ($CHCl_3$): 3600–2500 (br.), 2963*m*, 1728*m*, 1497*m*, 1457*m*, 1176*s*, 1124*s*, 1033*s*, 1010*s*. ^{1}H-NMR (400 MHz, CD_3OD): 0.87 (*d*, $J = 6.4$, Me); 0.89 (*d*, $J = 6.5$, Me); 1.30–1.39 (*m*, CH); 1.50–1.63 (*m*, CH_2); 2.35 (*s*, Me–C_6H_4); 2.81–2.88 (*m*, CHCO); 3.04 (*dd*, $J = 13.0$, 4.4, 1 H, CH_2N); 3.17 (*dd*, $J = 13.0$, 9.1, 1 H, CH_2N); 5.14 (*d*, $J = 12.2$, 1 H, CH_2O); 5.23 (*d*, $J = 12.2$, 1 H, CH_2O); 7.16–7.23 (*m*, 2 arom. H); 7.29–7.42 (*m*, 5 arom. H); 7.69–7.72 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 21.3, 22.3, 23.0 (Me); 26.9 (CH); 40.2, 41.9 (CH_2); 42.6 (CH); 68.2 (CH_2); 127.0, 129.5, 129.6, 129.7, 129.9 (CH); 137.1, 141.7, 143.6, 174.6 (C). EI-MS: 236 (2, $[M + H]^+$), 206 (17), 192 (7), 179 (96), 150 (31), 144 (30), 91 (100). Anal. calc. for $C_{21}H_{29}NO_5S$ (407.53): C 61.89, H 7.17, N 3.44; found: C 62.05, H 7.38, N 3.42.

(R)-2-{{[(tert-Butoxy)carbonyl]amino}methyl}-3-methylbutanoic Acid (Boc-(*R*)-β^2-HVal-OH; (*R*)-**13b**). The amino acid (*R*)-**11b** (2.10 g, 12.5 mmol) was dissolved in 1M NaOH (12.5 ml). A soln. of Boc_2O (2.86 g, 13.1 mmol) in 1,4-dioxane (1 ml) was added, and the biphasic system was vigorously stirred. From time to time, the pH of the mixture was adjusted to 9–10 by adding 1M NaOH. After 16 h, the mixture was diluted with Et_2O and washed with citric acid soln. and brine. The org. phase was dried (Na_2SO_4). Evaporation of the solvent gave (*R*)-**13b** (2.51 g, 87%). An anal. sample was purified by recrystallization from AcOEt/hexane. White solid. M.p. 71–72°. $[\alpha]_D^{r.t.} = -37.9$ ($c = 0.70$, AcOEt). IR ($CHCl_3$): 3600–2400(br.), 3456*m*, 2974*m*, 1708*s*, 1507*m*, 1368*m*, 1168*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.96 (*d*, $J = 6.8$, Me); 0.99 (*d*, $J = 6.8$, Me); 1.42 (*s*, *t*-Bu); 1.89 (*sext.*, $J = 6.8$, CH); 2.37 (*m*, CHCO); 3.20 (*dd*, $J = 9.2$, 13.6, 1 H, CH_2N); 3.27–3.32 (*m*, 1 H, CH_2N). ^{13}C-NMR (100 MHz, CD_3OD): 20.4, 20.7, 28.8 (Me); 29.8 (CH); 41.3 (CH_2); 53.6 (CH); 80.1, 158.3, 177.8 (C). EI-MS: 232 (8, $[M + 1]^+$), 193 (2), 176 (100), 158 (39). Anal. calc. for $C_{11}H_{21}NO_4$ (231.29): C 57.12, H 9.15, N 6.06; found: C 57.00, H 8.94, N 6.06.

Methyl (2S,3S)-3-{[(tert-Butoxy)carbonyl]amino}-2,4-dimethylpentanoate (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OMe; **14b**). LiBr (0.4 g, 4.59 mmol) was suspended in THF (5 ml). After cooling to −78° (i-$Pr)_2$NH (0.48 ml, 3.37 mmol) and BuLi (2.25 ml, 3.37 mmol) were added. After 15 min, a soln. of **10b** (0.375 g, 1.53 mmol) in THF (5 ml) was added during 10 min (clear soln.) and the mixture stirred for 2 h at −78°. MeI (0.38 ml, 6.12 mmol) was then added slowly (at −78°), and the mixture was stirred for 4 h at this temp., subsequently hydrolyzed with 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 (Et_2O/pentane 2:7) yielded the minor diastereoisomer **14b** as a colorless oil (0.136 g, 34%), the major diastereoisomer *epi*-**14b** as a colorless solid (0.22 g, 55%).

Data of **14b**: R_f 0.23 (Et_2O/pentane 2:7). $[\alpha]_D^{r.t.} = -34.9$ ($c = 1.0$, $CHCl_3$). IR (film): 3436*w*, 2968*s*, 2876*w*, 1720*s*, 1503*s*, 1461*m*, 1390*m*, 1366*m*, 1307*m*, 1237*m*, 1170*s*, 1100*m*, 1074*m*, 1041*m*, 985*w*, 914*w*, 870*w*, 831*w*, 759*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*t*, $J = 6.5$, 2 Me); 1.21 (*d*, $J = 7.1$, Me); 1.44 (*s*, 8.1 H, *t*-Bu, rotamer); 1.45 (*s*, 0.9 H, *t*-Bu, rotamer); 1.62–1.72 (*m*, Me_2C); 2.71–2.82 (*m*, CHCO); 3.38–3.49 (*m*, CHN); 3.67 (*s*, MeO); 4.80 (*d*, $J = 10.5$, 0.1 H, NH, rotamer); 5.22 (*d*, $J = 10.5$, 0.9 H, NH, rotamer). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.7, 19.1, 19.9, 28.4 (Me); 31.8, 40.6 (CH); 51.6 (Me); 58.6 (CH); 78.8, 156.4, 176.2 (C). EI-MS: 260 (13, $[M + 1]^+$),

246 (1), 216 (33), 204 (64), 186 (12), 172 (21), 160 (77), 130 (10), 116 (100), 84 (7), 72 (9), 57 (25), 41 (4). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.07, H 9.90, N 5.58.

Methyl (2R,3S)-3-{[(tert-Butoxy)carbonyl]amino}-2,4-dimethylpentanoate (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OMe; *epi*-**14b**). BuLi (32 ml, 44.8 mmol) was added to a soln. of $(i\text{-Pr})_2NH$ (6.4 ml, 44.8 mmol) and DMPU (9.9 ml, 81.6 mmol) in THF (160 ml) at −78°. After 15 min, a soln. of **10b** (5.0 g, 20.4 mmol) in THF (20 ml) was added to the clear yellow soln. during 10 min and the mixture stirred for 1 h at −78°. MeI (5.1 ml, 81.6 mmol) was then added slowly (at −78°), and the mixture was stirred for 3 h at this temp., subsequently hydrolyzed with 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 (Et_2O/pentane 1:5) yielded the major diastereoisomer *epi*-**14b** as a colorless solid (2.99 g, 58%), the minor diastereoisomer **14b** as a colorless oil (1.11 g, 21%) and mixed fractions (0.58 g, 11%). Total yield: 4.68 g (90%).

Data of epi-**14b**: M.p. 47–50°. R_f 0.12 (Et_2O/pentane 2:7). $[\alpha]_D^{r.t.} = -16.3$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 2970*m*, 1723*s*, 1713*s*, 1503*s*, 1456*m*, 1436*w*, 1392*m*, 1365*s*, 1179*s*, 1063*w*, 991*w*, 865*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, $J = 6.8$, Me); 0.94 (*d*, $J = 6.7$, Me); 1.12 (*d*, $J = 7.0$, Me); 1.43 (*s*, 7.6 H, *t*-Bu, rotamer); 1.47 (*s*, 1.4 H, *t*-Bu, rotamer); 1.63–1.71 (*m*, Me_2CH); 2.54–2.66 (*m*, CHCO); 3.67 (*s*, MeO); 3.76–3.85 (*m*, CHN); 4.38 (*d*, $J = 10.4$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.2, 17.5, 20.2, 28.4 (Me); 30.4, 42.3 (CH); 51.8 (Me); 57.4 (CH); 79.2, 155.9, 175.3 (C). EI-MS: 260 (7, $[M + 1]^+$), 216 (42), 204 (34), 186 (12), 172 (29), 160 (71), 130 (11), 116 (100), 72 (23), 57 (57), 41 (9). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.01, H 9.82, N 5.38.

Methyl (2S,3S)-3-{[(tert-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OMe; **14c**). LiCl (0.29 g, 6.87 mmol) was suspended in THF (7 ml). After cooling to −78°, $(i\text{-Pr})_2NH$ (0.72 ml, 5.04 mmol) and BuLi (3.36 ml, 5.04 mmol) were added. After 15 min, a soln. of **10b** (0.593 g, 2.29 mmol) in THF (5 ml) was added during 20 min (clear soln.) and the mixture stirred for 1 h at −78°. MeI (0.57 ml, 9.16 mmol) was then added slowly (at −78°), and the mixture was stirred for 18 h allowing to reach −56°, subsequently hydrolyzed with 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 (AcOEt/pentane 1:5) yielded the major diastereoisomer **14c** as a colorless oil (0.348 g, 56%), the minor diastereoisomer *epi*-**14c** as a colorless waxy solid (0.117 g, 19%).

Data of **14c**: R_f 0.49 (AcOEt/pentane 1:5). $[\alpha]_D^{r.t.} = -43.0$ ($c = 1.0$, $CHCl_3$). IR (film): 3369*m*, 2956*s*, 2871*m*, 1740*s*, 1702*s*, 1523*s*, 1456*m*, 1436*m*, 1390*m*, 1366*s*, 1324*m*, 1301*m*, 1251*s*, 1174*s*, 1102*m*, 1072*m*, 1038*m*, 1023*m*, 999*m*, 948*w*, 914*w*, 873*w*, 757*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*t*, $J = 6.0$, 2 Me); 1.13–1.21 (*m*, CH, Me); 1.31–1.38 (*m*, CH); 1.44 (*s*, 8 H, *t*-Bu, rotamer); 1.47 (*s*, 1 H, *t*-Bu, rotamer); 1.60–1.70 (*m*, CH); 2.59–2.69 (*m*, CHCO); 3.68 (*s*, MeO); 3.70–3.87 (*m*, CHN); 4.70 (*d*, $J = 9.8$, 0.1 H, NH, rotamer); 5.02 (*d*, $J = 9.8$, 0.9 H, NH, rotamer). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.3, 22.1, 23.1 (Me); 24.9 (CH); 28.4 (Me); 43.0 (CH_2); 43.3, 50.7 (CH); 51.5 (Me); 78.9, 155.9, 175.8 (C). EI-MS: 273 (1, M^+), 216 (15), 200 (15), 186 (38), 172 (5), 160 (41), 144 (34), 130 (74), 116 (99), 100 (15), 86 (100), 70 (6), 57 (21). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.66, H 9.74, N 5.08.

Methyl (2R,3S)-3-{[(tert-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OMe; *epi*-**14c**). BuLi (30.9 ml, 39.0 mmol) was added to a soln. of $(i\text{-Pr})_2NH$ (5.56 ml, 39.0 mmol) and DMPU (8.56 ml, 70.8 mmol) in THF (120 ml) at −78°. After 15 min, a soln. of Boc-(*S*)-β^3-HLeu-OMe (**10c**; 4.6 g, 17.7 mmol) in THF (18 ml) was added to the clear yellow soln. during 10 min and the mixture stirred for 1 h at −78°. MeI (4.41 ml, 70.8 mmol) was then added slowly (at −78°), and the mixture was stirred for 3 h at this temp., subsequently hydrolyzed with 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 (Et_2O/pentane 1:6) yielded the major diastereoisomer *epi*-**14c** as a colorless solid (2.72 g, 57%), the minor diastereoisomer **14c** as a colorless oil (1.29 g, 27%). Total yield: 4.01 g (84%).

Data of epi-**14c**: M.p. 41–43°. R_f 0.40 (AcOEt/pentane 1:5). $[\alpha]_D^{r.t.} = -46.1$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 2958*m*, 1708*s*, 1503*s*, 1456*m*, 1436*w*, 1392*m*, 1368*s*, 1171*s*, 1101*w*, 909*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (2*s*, $J = 6.7$, 6.5, 2 Me); 1.14 (*d*, $J = 7.2$, Me); 1.18–1.35 (*m*, CH_2); 1.43 (*s*, *t*-Bu); 1.59–1.68 (Me_2CH); 2.50–2.64 (*m*, CHCO); 3.69 (*s*, MeO); 3.87–3.94 (*m*, CHN); 4.62 (*d*, $J = 9.5$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.9, 21.7, 23.5 (Me); 25.0 (CH); 28.4 (Me); 41.2 (CH_2); 44.3, 50.8 (CH); 51.7 (Me); 79.2, 155.5, 175.1 (C). EI-MS: 274 (3, $[M + 1]^+$), 218 (31), 200 (10), 186 (37), 174 (23), 160 (19), 144 (21), 130 (100), 116 (51), 97 (13), 88 (30), 86 (97), 57 (42). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.47, H 10.02, N 5.23.

Benzyl (2S,3S)-3-{[(tert-Butoxy)carbonyl]amino}-2,4-dimethylpentanoate (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OBn; **15b**). Compound **14b** (0.90 g, 3.47 mmol) was transesterified with 0.7 equiv. of $Ti(OBn)_4$ for 40 h according to *GP 7*. FC (Et_2O/pentane 1:5) yielded **15b** (0.93 g, 80%). Colorless oil. R_f 0.32 (Et_2O/pentane 1:5). $[\alpha]_D^{r.t.} = -30.5$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 2974*m*, 2933*m*, 2875*w*, 1720*s*, 1707*s*, 1502*s*, 1455*m*, 1392*m*,

1368*m*, 1165*s*, 1019*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*t*, *J* = 6.9, 2 Me); 1.23 (*d*, *J* = 7.1, Me); 1.43 (*s*, *t*-Bu); 1.59–1.70 (*m*, CH); 2.73 (*m*, 0.1 H, CHCO, rotamer); 2.80–2.87 (*m*, 0.9 H, CHCO, rotamer); 3.33–3.39 (*m*, 0.1 H, CHN, rotamer); 3.40–3.44 (*m*, 0.9 H, CHN, rotamer); 4.84 (*d*, *J* = 10.6, 0.1 H, NH, rotamer); 5.09 (*d*, *J* = 12.4, 1 H, PhCH_2); 5.13 (*d*, *J* = 12.4, 1 H, PhCH_2); 5.24 (*d*, *J* = 10.4, 0.9 H, NH, rotamer); 7.30–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.70, 19.17, 19.94, 28.42 (Me); 31.85, 40.60, 58.69 (CH); 66.30 (CH_2); 78.79 (C); 128.10, 128.31, 128.61 (CH); 135.79, 156.39, 175.61 (C). EI-MS: 336 (3, $[M + 1]^+$), 326 (2), 292 (14), 262 (5), 248 (2), 236 (48), 186 (1), 172 (6), 116 (33), 91 (100), 83 (4), 72 (9), 57 (12). Anal. calc. for $C_{19}H_{29}NO_4$ (335.44): C 68.03, H 8.71, N 4.18; found: C 68.18, H 8.55, N 4.14.

*Benzyl (2S,3S)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate* (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OBn; **15c**). Compound **14c** (59 mg, 0.21 mmol) was transesterified with 4 equiv. of $Ti(OBn)_4$ for 4 d according to *GP 7*. FC (Et_2O/pentane 1:6 to 1:3) yielded **15c** (51 mg, 69%). Colorless solid. M.p. 43–44° (pentane). R_f 0.22 (Et_2O/pentane 1:6). $[\alpha]_D^{r.t.} = -24.9$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 2961*m*, 1706*s*, 1503*s*, 1456*m*, 1392*m*, 1367*m*, 1164*s*, 1116*w*, 1028*w*, 626*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86 (*d*, *J* = 6.7, 2 Me); 1.09–1.18 (*m*, CH); 1.21 (*d*, *J* = 7.2, Me); 1.26–1.38 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.58–1.65 (*m*, CH); 2.59–2.73 (*m*, CHCO); 3.74–3.88 (*m*, CHN); 5.02 (*d*, *J* = 10.0, NH); 5.08 (*d*, *J* = 12.3, 1 H, PhCH_2); 5.16 (*d*, *J* = 12.3, 1 H, PhCH_2). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.4, 22.1, 23.0 (Me); 24.9 (CH); 28.4 (Me); 43.0 (CH_2); 43.2, 50.7 (CH); 66.2 (CH_2); 78.9 (C); 128.2, 128.3, 128.6 (CH); 135.9, 155.9, 175.2 (C). EI-MS: 340 (< 1, $[M + 1]^+$), 292 (2), 276 (1), 250 (2), 236 (7), 192 (24), 169 (4), 158 (2), 144 (5), 130 (46), 107 (13), 91 (100), 57 (8). Anal. calc. for $C_{20}H_{31}NO_4$ (349.47): C 68.74, H 8.94, N 4.01; found: C 68.65, H 8.83, N 3.97.

*(2S,3S)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethylpentanoic Acid* (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH; **16b**). Compound **15b** (0.476 g, 1.42 mmol) was debenzylated in MeOH according to *GP 6*. **16b** (0.347 g, 99%). Colorless needles. M.p. 88–90°. R_f 0.42 ($CHCl_3$/MeOH 9:1). $[\alpha]_D^{r.t.} = -19.0$ (*c* = 1.0, MeOH). IR ($CHCl_3$): 3438*w*, 2981*m*, 2926*w*, 2873*w*, 2672*w*, 1706*s*, 1502*m*, 1461*w*, 1413*w*, 1392*m*, 1368*m*, 1306*w*, 1172*m*, 974*w*, 897*w*, 868*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88 (*d*, *J* = 6.7, Me); 0.94 (*d*, *J* = 6.7, Me); 1.16 (*d*, *J* = 7.1, Me); 1.44 (*s*, *t*-Bu); 1.72–1.82 (*m*, CH); 2.63–2.74 (*m*, CHCO); 3.38–3.43 (*m*, CHN). ^{13}C-NMR (100 MHz, CD_3OD): 15.82, 18.75, 20.50, 28.80 (Me); 32.11, 42.45, 59.73 (CH); 79.99, 158.51, 179.11 (C). FAB-MS: 513 (6, $[2M + Na]^+$), 491 (33, $[2M + 1]^+$), 268 (12), 246 (38, $[M + 1]^+$), 202 (6), 190 (100), 172 (33), 154 (11), 146 (27), 136 (12), 116 (19), 101 (10), 91 (15).

Methyl (2R,3S)-3-(Benzoylamino)-2,4-dimethylpentanoate (Bz-(2*R*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OMe; *epi*-**17b**). Compound *epi*-**14b** (0.309 g, 1.19 mmol) was transformed according to *GP 1*. FC (AcOEt/pentane 1:3) yielded *epi*-**17b** (0.286 g, 92%). White solid. M.p. 89–90°. R_f 0.36 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -16.6$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3442*w*, 3007*m*, 2969*m*, 1732*s*, 1665*s*, 1602*w*, 1580*w*, 1514*s*, 1487*s*, 1436*w*, 1311*w*, 1269*m*, 1141*w*, 1063*w*, 1029*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.96 (*d*, *J* = 6.8, Me); 1.01 (*d*, *J* = 6.7, Me); 1.21 (*d*, *J* = 7.1, Me); 1.79–1.90 (*m*, Me_2CH); 2.78 (*q*, *J* = 7.0, CHCO); 3.70 (*s*, MeO); 4.36–4.41 (*m*, CHN); 6.10 (br. *d*, *J* = 10.2, NH); 7.41–7.56 (*m*, 3 arom. H); 7.74–7.96 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.5, 17.7, 20.4 (Me); 30.3, 42.1 (CH); 51.9 (Me); 56.1, 126.9, 128.7, 131.4 (CH); 135.0, 167.5, 175.2 (C). EI-MS: 263 (< 1, $[M + 1]^+$), 232 (7), 220 (95), 188 (4), 176 (36), 105 (100). Anal. calc. for $C_{15}H_{21}NO_3$ (263.34): C 68.42, H 8.04, N 5.32; found: C 68.50, H 7.92, N 5.37.

Methyl (2S,3S)-3-(Benzoylamino)-2,5-dimethylhexanoate (Bz-(*S*,*S*)-$\beta^{2,3}$-HLeu(α-Me)-OMe; **17c**). Compound **14c** (0.237 g, 0.87 mmol) was transformed according to *GP 1*. FC (AcOEt/pentane 1:4) yielded **17c** (0.175 g, 72%). Colorless crystalline solid. M.p. 84–86° (pentane). R_f 0.27 (AcOEt/pentane 1:4). $[\alpha]_D^{r.t.} = -59.1$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3427*w*, 3004*m*, 3957*m*, 2871*w*, 1718*s*, 1655*s*, 1580*w*, 1520*s*, 1487*s*, 1462*m*, 1437*w*, 1384*w*, 1366*w*, 1178*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.7, Me); 0.98 (*d*, *J* = 6.5, Me); 1.26 (*d*, *J* = 7.2, Me); 1.28–1.34 (*m*, CH); 1.47–1.54 (*m*, CH); 1.62–1.75 (*m*, CH); 2.77 (*dq*, *J* = 14.4, 7.2, 3.5, CHCO); 3.73 (*s*, MeO); 4.38–4.45 (*m*, *J* = 9.7, 4.9, 3.5, CHN); 7.06 (*d*, *J* = 9.5, NH); 7.42–7.52 (*m*, 3 arom. H); 7.79–7.84 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.5, 22.3, 23.1 (Me); 25.1, 43.0 (CH); 43.6 (CH_2); 49.7 (CH); 51.8 (Me); 126.9, 128.6, 131.4 (CH); 134.7, 167.1, 176.8 (C). EI-MS: 278 (16, $[M + 1]^+$), 246 (7), 221 (38), 190 (49), 172 (4), 116 (12), 105 (100), 77 (19). Anal. calc. for $C_{16}H_{23}NO_3$ (277.36): C 69.29, H 8.36, N 5.05; found: C 69.24, H 8.33, N 5.09.

(2R,3S)-3-(Benzylamino)-2,4-dimethylpentan-1-ol (*epi*-**18b**). Compound *epi*-**17b** (0.229 g, 0.87 mmol), dissolved in THF (3 ml), was added under Ar to a suspension of $LiAlH_4$ (0.116 g, 3.0 mmol) in THF (7 ml). The resulting light-yellow soln. was heated to reflux for 3 h and hydrolyzed subsequently with H_2O (5 ml). After filtration through *Celite*, it was extracted with AcOEt and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the crude product *epi*-**18b** (0.123 g, 67%) as a light-yellow waxy solid which was used for the following cyclization without further purification. R_f 0.21 ($CHCl_3$/MeOH/Et_3N 18:1:0.1). ^{1}H-NMR (200 MHz, $CHCl_3$): 0.94 (*d*, *J* = 3.2, Me); 0.98 (*d*, *J* = 3.3, Me); 1.07 (*d*, *J* = 6.6, Me); 1.86–2.01 (*m*, MeCH, Me_2CH); 2.57

(*dd*, J = 7.1, 2.9, CHN); 3.7 (br. *s*, NH, OH); 3.70 (*dd*, J = 10.4, 5.8, 1 H, CH_2O); 3.81 (*dd*, J = 10.4, 2.9, 1 H, CH_2O); 3.87 (*s*, PhCH_2); 7.26–7.34 (*m*, 5 arom. H). EI-MS: 222 (3, $[M + 1]^+$), 203 (7), 192 (2), 162 (40), 146 (2), 91 (19).

(2S,3S)-3-(Benzylamino)-2,5-dimethylhexan-1-ol (**18c**). Compound **17c** (0.124 g, 0.45 mmol), dissolved in THF (2 ml), was added under Ar to a suspension of $LiAlH_4$ (0.061 g, 1.61 mmol) in THF (2 ml). The resulting yellow soln. was heated to reflux for 2.5 h and hydrolyzed subsequently with H_2O (5 ml). After filtration through *Celite*, it was extracted with AcOEt and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the crude product **18c** (0.106 g, quant.) as a colorless oil, which was used for the following cyclization without further purification. R_f 0.30 ($CHCl_3$/MeOH/Et_3N 9:1:0.5). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.95 (*m*, 3 Me); 1.46–1.86 (*m*, CH_2, Me_2CH, MeCH); 2.72 (*dd*, J = 13.3, 6.2, CHN); 3.54 (*dd*, J = 10.8, 7.5, 1 H, CH_2O); 3.81 (*d*, J = 12.5, 1 H, PhCH_2); 3.82 (*dd*, J = 10.8, 3.3, 1 H, CH_2O); 3.96 (*d*, J = 12.5, 1 H, PhCH_2); 4.44 (br. *s*, NH, OH); 7.29–7.37 (*m*, 5 arom. H). EI-MS: 236 (1, $[M + 1]^+$), 204 (4), 190 (2), 176 (100), 133 (2), 106 (1), 91 (28).

(4S,5S)-3-Benzyl-4-isobutyl-5-methyl-1,3-oxazinan-2-one (**19**). To a soln. of **18c** (44 mg, 0.19 mmol) in CH_2Cl_2/THF 1:1 (3 ml) was added Et_3N (52 µl, 0.37 mmol). The mixture was cooled to −50°, and a soln. of triphosgene (18 mg, 0.062 mmol) in THF (1.5 ml) was added. The mixture was allowed to warm to r.t. within 3 h and diluted with Et_2O. The salts were filtered off, and it was evaporated to yield a yellow oil. FC (AcOEt/pentane 1:3 → 1:1) yielded **19** (16.9 mg, 35%). Colorless solid. Crystallization from pentane gave single crystals suitable for X-ray analysis. M.p. 95–96° (pentane). R_f 0.13 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -40.8$ (c = 0.5, $CHCl_3$). IR ($CHCl_3$): 3008*m*, 2961*m*, 2932*m*, 2872*w*, 1676*s*, 1484*m*, 1451*m*, 1370*w*, 1311*w*, 1256*m*, 1152*m*, 1080*w*, 1011*w*, 960*w*, 829*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.86 (*d*, J = 6.1, Me); 0.89 (*d*, J = 7.1, Me); 0.94 (*d*, J = 6.3, Me); 1.44–1.59 (*m*, CH_2, Me_2CH); 1.85–1.91 (*m*, MeC*H*); 2.90–2.92 (*m*, CHN); 3.90 (*d*, J = 14.9, 1 H, PhCH_2); 3.94 (*dt*, J = 11.2, 1.9, 1 H, CH_2O); 4.42 (*dd*, J = 11.1, 3.0, 1 H, CH_2O); 10.43 (*d*, J = 14.9, 1 H, PhCH_2); 7.26 (*m*, 5 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 16.21, 21.64, 23.76 (Me); 25.20, 28.64 (CH); 42.26, 50.13 (CH_2); 57.41 (CH); 67.80 (CH_2); 127.69, 128.56, 128.61 (CH); 137.31, 153.63 (C). NOE (300 MHz, $CDCl_3$): Irradiation at 1.88: strong positive NOE at 0.9 and positive NOE at 2.91, 3.94, and 4.42; irradiation at 2.91: strong positive NOE at 0.9 and positive NOE at 3.94 and 1.88; irradiation at 4.42: strong positive NOE at 3.94 and positive NOE at 1.88. EI-MS: 261 (20, M^+), 218 (4), 204 (70), 176 (8), 160 (14), 150 (13), 133 (6), 114 (15), 91 (100).

(4S,5R)-3-Benzyl-4-isopropyl-5-methyl-1,3-oxazinan-2-one (**20**). To a soln. of *epi*-**18b** (60 mg, 0.27 mmol) in CH_2Cl_2/THF 1:1 (2 ml) at 0° was added Et_3N (75 µl, 0.54 mmol), followed by a soln. of triphosgene (30 mg, 0.1 mmol) in THF (1 ml). The mixture was stirred for 2.5 h at 0°, then diluted with Et_2O, filtered, and evaporated. The resulting yellow oil was dissolved in AcOEt, washed with citric acid soln. (pH 2.5) and sat. NaCl soln., dried ($MgSO_4$), and evaporated. FC (AcOEt/pentane 2:3) yielded **20** (24 mg, 18%). Colorless solid. Crystallization from hexane gave single cristals suitable for X-ray analysis. M.p. 94–95° (hexane). R_f 0.29 (AcOEt/pentane 2:3). $[\alpha]_D^{r.t.} = -50.0$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2968*m*, 1676*s*, 1515*w*, 1484*s*, 1451*s*, 1394*w*, 1359*w*, 1248*m*, 1153*m*, 1080*w*, 1034*w*, 967*w*, 879*w*, 658*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, J = 7.0, Me); 1.06 (*d*, J = 7.1, Me); 1.11 (*d*, J = 7.1, Me); 1.96–2.08 (*m*, Me_2CH); 2.17–2.28 (*m*, MeC*H*); 3.02–3.04 (*m*, CHN); 3.82 (*d*, J = 15.2, 1 H, PhCH_2); 4.07 (*t*, J = 11.5, 1 H, CH_2O); 4.16 (*ddd*, J = 11.0, 5.5, 1.7, 1 H, CH_2O); 5.43 (*d*, J = 15.2, 1 H, PhCH_2); 7.26–7.36 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.93, 19.59, 24.61 (Me); 28.04, 32.36 (CH); 53.19 (CH_2); 61.96 (CH); 69.84 (CH_2); 127.62, 127.99, 128.70 (CH); 136.98, 153.93 (C). NOE (300 MHz, $CDCl_3$): Irradiation at 2.04: strong positive NOE at 4.07, 1.06, and 1.11; irradiation at 2.23: strong positive NOE at 3.03 and 4.16; irradiation at 3.03: positive NOE at 2.23. EI-MS: 247 (2, M^+), 204 (18), 160 (3), 117 (2), 104 (2), 91 (100), 77 (2), 65 (6), 56 (2). Anal. calc. for $C_{15}H_{21}NO_2$ (247.34): C 72.84, H 8.56, N 5.66; found: C 72.66, H 8.63, N 5.71.

(4S)-3-[(2R)-3-(Benzoylamino)-2-methyl-1-oxopropyl]-4-(phenylmethyl)oxazolidin-2-one ((4*S*,2′*R*)-**22a**). According to *GP 2*, (*S*)-**21a** (4.70 g, 20 mmol) was treated with $TiCl_4$ (2.20 ml, 20 mmol), Et_3N (2.80 ml, 20 mmol), and N-(chloromethyl)benzamide (4.07 g, 24 mmol). Recrystallization from AcOEt/hexane gave (4*S*,2′*R*)-**22a** (6.26 g, 85%). White needles. M.p. 120–121°. $[\alpha]_D^{r.t.} = +27.0$ (c = 0.89, $CHCl_3$). IR ($CHCl_3$): 3454*m*, 3008*m*, 1782*s*, 1690*m*, 1660*s*, 1580*m*, 1522*s*, 1488*m*, 1456*m*, 1388*s*, 1351*m*, 1289*m*, 1096*m*, 1015*m*, 968*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.28 (*d*, J = 7.0, Me); 2.71 (*dd*, J = 13.5, 9.6, 1 H, PhCH_2); 3.27 (*dd*, J = 13.4, 3.4, 1 H, PhCH_2); 3.69–3.75 (*m*, 1 H, CH_2N); 3.77–3.84 (*m*, 1 H, CH_2N); 4.02–4.10 (*m*, CHCO); 4.15–4.24 (*m*, CH_2O); 4.67 (*dddd*, J = 9.6, 7.6, 3.4, 2.8, CHN); 6.81 (br., NH); 7.14–7.29 (*m*, 5 arom. H); 7.41–7.52 (*m*, 3 arom. H); 7.77–7.79 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.0 (Me); 37.8 (CH_2); 38.2 (CH); 42.4 (CH_2); 55.5 (CH); 66.3 (CH_2); 126.9, 127.4, 128.6, 128.9, 129.3, 131.5 (CH); 134.3, 135.1, 153.2, 167.2, 175.9 (C). FAB-MS: 733 (20, $[2M + 1]^+$), 367 (100, $[M + 1]^+$), 307 (14), 190 (88), 154 (65), 137 (56), 105 (87). Anal. calc. for $C_{21}H_{22}N_2O_4$ (366.42): C 68.84, H 6.05, N 7.65; found: C 68.90, H 5.97, N 7.64.

(4R)-3-[(2S)-3-(Benzoylamino)-2-methyl-1-oxopropyl]-4-(phenylmethyl)oxazolidin-2-one ((4*R*,2′*S*)-**22a**). According to *GP 2*, (*R*)-**21a** (4.70 g, 20 mmol) was treated with $TiCl_4$ (2.20 ml, 20 mmol), Et_3N (2.80 ml, 20 mmol), and *N*-(chloromethyl)benzamide (4.57 g, 27 mmol). Recrystallization from AcOEt/hexane gave (4*R*,2′*S*)-**22a** (4.03 g, 55%). White needles. M.p. 118–119°. $[\alpha]_D^{r.t.} = -24.5$ (*c* = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to those of (4*S*,2′*R*)-**22a**.

(4S)-3-{(2R)-2-[(Benzoylamino)methyl]-3-methyl-1-oxobutyl}-4-(phenylmethyl)oxazolidin-2-one ((4*S*,2′*R*)-**22b**). According to *GP 2* (*S*)-**21b** (5.23 g, 20 mmol) was treated with $TiCl_4$ (2.20 ml, 20 mmol), Et_3N (2.80 ml, 20 mmol), and *N*-(chloromethyl)benzamide (2.80 g, 17 mmol). FC (Et_2O/pentane 2:1, then Et_2O) gave (4*S*,2′*R*)-**22b** (6.38 g, 81%). Colorless foam. R_f 0.58 (Et_2O). $[\alpha]_D^{r.t.} = +65.6$ (*c* = 1.13, $CHCl_3$). IR ($CHCl_3$): 3446*m*, 3007*m*, 2968*m*, 1781*s*, 1662*s*, 1603*m*, 1580*m*, 1523*s*, 1487*s*, 1387*s*, 1349*m*, 1290*m*, 1102*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.02 (*d*, *J* = 6.4, Me); 1.04 (*d*, *J* = 6.3, Me); 2.11–2.20 (*m*, Me_2CH); 2.70 (*dd*, *J* = 9.4, 13.5, 1 H, $PhCH_2$); 3.25 (*dd*, *J* = 3.5, 13.5, 1 H, $PhCH_2$); 3.64–3.69 (*m*, CHCO); 3.90–4.02 (*m*, CH_2N); 4.12–4.23 (*m*, CH_2O); 4.66–4.72 (*m*, CHN); 6.80 (br., NH); 7.12–7.26 (*m*, 5 arom. H); 7.41–7.52 (*m*, 3 arom. H); 7.76–7.79 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.3, 21.0 (Me); 28.8 (CH); 37.8, 39.1 (CH_2); 48.8, 55.6 (CH); 66.2 (CH_2); 126.9, 127.3, 128.6, 128.9, 129.3, 131.5 (CH); 134.2, 135.0, 153.5, 167.0, 175.6 (C). FAB-MS: 789 (24, $[2M + 1]^+$), 395 (100, $[M + 1]^+$), 307 (17), 218 (90), 154 (65), 105 (78). Anal. calc. for $C_{23}H_{26}N_2O_4$ (394.47): C 70.03, H 6.64, N 7.10; found: C 70.04, H 6.70, N 7.10.

(4R)-3-{(2S)-2-[(Benzoylamino)methyl]-3-methyl-1-oxobutyl}-4-(phenylmethyl)oxazolidin-2-one ((4*R*,2′*S*)-**22b**). According to *GP 2*, (*R*)-**21b** (3.65 g, 14 mmol) was treated with $TiCl_4$ (1.53 ml, 14 mmol), Et_3N (1.95 ml, 14 mmol), and *N*-(chloromethyl)benzamide (3.20 g, 18.9 mmol). FC (Et_2O) gave (4*R*,2′*S*)-**22b** (4.14 g, 81%). Colorless foam. R_f 0.60 (Et_2O). $[\alpha]_D^{r.t.} = -63.3$ (*c* = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to those of (4*S*,2′*R*)-**22b**.

(4S)-3-{(2S)-2-[(Benzoylamino)methyl]-4-methyl-1-oxopentyl}-4-(phenylmethyl)oxazolidin-2-one ((4*S*,2′*R*)-**22c**). According to *GP 2*, (*S*)-**21c** (5.52 g, 20 mmol) was treated with $TiCl_4$ (2.20 ml, 20 mmol), Et_3N (2.80 ml, 20 mmol), and *N*-(chloromethyl)benzamide (4.07 g, 24 mmol). FC (Et_2O/pentane 2:1) gave (4*R*,2′*S*)-**22c** (6.39 g, 78%). Colorless foam. R_f 0.30 (Et_2O/pentane 2:1). $[\alpha]_D^{r.t.} = +37.4$ (*c* = 0.50, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2961*m*, 1778*s*, 1662*m*, 1521*m*, 1487*m*, 1387*s*, 1351*m*, 1289*m*, 1104*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.91 (*d*, *J* = 6.5, Me); 0.95 (*d*, *J* = 6.4, Me); 1.36–1.50 (*m*, CH); 1.62–1.80 (*m*, CH_2); 2.65 (*dd*, *J* = 13.5, 9.6, 1 H, $PhCH_2$); 3.25 (*dd*, *J* = 13.4, 3.4, 1 H, $PhCH_2$); 3.65 (*ddd*, *J* = 13.6, 4.2, 4.0, CHCO); 3.84–3.94 (*m*, 1 H, CH_2N); 4.12–4.24 (*m*, 3 H, CH_2O, CH_2N); 4.63–4.70 (*m*, CHN); 6.87 (br., NH); 7.12–7.28 (*m*, 5 arom. H); 7.40–7.53 (*m*, 3 arom. H); 7.76–7.80 (*m*, 2 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.2, 22.8 (Me); 25.9 (CH); 37.8, 38.7 (CH_2); 41.0 (CH); 41.4 (CH_2); 55.7 (CH); 66.3 (CH_2); 126.9, 127.3, 128.6, 128.9, 129.3, 131.5 (CH); 134.2, 135.1, 153.5, 167.1, 175.7 (C). FAB-MS: 1226 (1, $[3M + 1]^+$), 817 (21, $[2M + 1]^+$), 409 (97, $[M + 1]^+$), 232 (100), 154 (25), 105 (83). Anal. calc. for $C_{24}H_{28}N_2O_4$ (408.50): C 70.57, H 6.91, N 6.86; found: C 70.46, H 6.78, N 6.98.

(4R)-3-{(2S)-[(Benzoylamino)methyl]-4-methyl-1-oxopentyl}-4-(phenylmethyl)oxazolidin-2-one ((4*R*,2′*S*)-**22c**). According to *GP 2*, (*R*)-**21c** (15.2 g, 55.4 mmol) was treated with $TiCl_4$ (6.1 ml, 55.6 mmol), Et_3N (7.8 ml, 55.9 mmol), and *N*-(chloromethyl)benzamide (11.65 g, 68.7 mmol). FC (Et_2O/pentane 1:1) gave (4*R*,2′*S*)-**22c** (16.78 g, 74%). Colorless foam. Spectroscopic data: corresponding to those of (4*S*,2′*R*)-**22c**, but with opposite sign of optical rotation.

(4R)-3-{(2S)-[(Benzoylamino)methyl]-3-phenyl-1-oxopropyl}-4-(phenylmethyl)oxazolidin-2-one ((4*R*,2′*S*)-**22d**). According to *GP 2*, (*R*)-**21d** (4.33 g, 14 mmol) was treated with $TiCl_4$ (1.54 ml, 14 mmol), Et_3N (1.95 ml, 14 mmol), and *N*-(chloromethyl)benzamide (3.08 g, 18.2 mmol). FC (Et_2O/hexane 8:2) gave (4*S*,2′*R*)-**22d** (3.85 g, 62%). Colorless foam. R_f 0.50 (Et_2O). $[\alpha]_D^{r.t.} = -79.8$ (*c* = 1.38, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3005*m*, 1774*s*, 1697*m*, 1662*s*, 1603*w*, 1581*w*, 1522*m*, 1487*m*, 1455*w*, 1387*m*, 1351*m*, 1291*w*, 1261*m*, 1110*m*, 1013*m*, 967*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.68 (*dd*, *J* = 9.5, 13.7, 1 H, $PhCH_2CHN$); 2.92 (*dd*, *J* = 7.4, 13.3, 1 H, $PhCH_2CHCO$); 3.11 (*dd*, *J* = 8.3, 13.9, 1 H, $PhCH_2CHCO$); 3.23 (*dd*, *J* = 3.3, 13.3, 1 H, $PhCH_2CHN$); 3.69 (*ddd*, *J* = 13.7, 4.6, 4.6, CHCO); 3.88–4.10 (*m*, 3 H, CH_2N, CH_2O); 4.39–4.52 (*m*, 2 H, CH_2O, CHN); 6.79 (br., NH); 7.11–7.34 (*m*, 10 arom. H); 7.39–7.52 (*m*, 3 arom. H); 7.74–7.78 (*m*, 2 arom. H). ^{13}C-NMR (50 MHz, CD_3OD): 34.1, 35.0 (CH_2); 38.8 (CH); 42.2 (CH_2); 53.2 (CH); 63.9 (CH_2); 124.4, 124.6, 125.0, 126.3, 126.6, 126.8, 127.0, 129.2, 131.9 (CH); 132.8, 135.7 (C); 151.5, 164.9, 172.5 (C). FAB-MS: 1327 (< 1, $[3M + 1]^+$), 885 (11, $[2M + 1]^+$), 443 (100, $[M + 1]^+$). Anal. calc. for $C_{27}H_{26}N_2O_4$ (442.51): C 73.29, H 5.92, N 6.33; found: C 72.99, H 6.16, N 6.29.

(R)-3-(Benzoylamino)-2-methylpropanoic Acid (Bz-(*R*)-β^2-HAla-OH; (*R*)-**23a**). According to *GP 3*, (4*S*,2′*R*)-**22a** (3.50 g, 9.6 mmol) was treated with 30% H_2O_2 soln. (4.10 ml, 40 mmol) and LiOH · H_2O (667 mg, 16 mmol). Recrystallization from AcOEt/hexane gave (*R*)-**23a** (1.79 g, 90%). White crystals. M.p. 111–112°. $[\alpha]_D^{r.t.} = -25.2$ (*c* = 1.23, $CHCl_3$). IR ($CHCl_3$): 3600–2400 (br.), 3450*m*, 3008*m*, 1712*s*, 1655*s*, 1579*m*, 1523*s*,

1488*s*, 1465*m*, 1287*m*, 1041*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.26 (*d*, 7.3, Me); 2.85 (*m*, CHCO); 3.51 (*m*, 1 H, CH_2N); 3.73 (*m*, 1 H, CH_2N); 6.99 (*t*, *J* = 5.9, NH); 7.36–7.51 (*m*, 3 arom. H); 7.73–7.76 (*m*, 2 arom. H); 10.3 (br., COOH). ^{13}C-NMR (75 MHz, $CDCl_3$): 14.8 (Me); 39.3 (CH); 42.0 (CH_2); 127.0; 128.6; 131.7 (CH); 134.0; 168.1; 180.0 (C). EI-MS: 207 (49, M^+), 190 (7), 161 (29), 134 (16), 105 (100), 77 (14). Anal. calc. for $C_{11}H_{13}NO_3$ (207.23): C 63.76, H 6.32, N 6.76; found: C 63.98, H 6.10, N 6.48.

(S)-3-(Benzoylamino)-2-methylpropanoic Acid (Bz-(*S*)-β^2-HAla-OH; (*S*)-**23a**). According to *GP 3*, (4*R*,2′*S*)-**22a** (3.87 g, 10.6 mmol) was treated with 30% H_2O_2 soln. (4.30 ml, 42 mmol) and LiOH · H_2O (711 mg, 17 mmol). Recrystallization from AcOEt/hexane gave (*S*)-**23a** (1.96 g, 90%). White crystals. R_f 0.44 (AcOEt/hexane/AcOH 5:5:0.5). M.p. 110°, $[\alpha]_D^{r.t.} = +25.4$ (*c* = 1.18, $CHCl_3$). Other spectroscopic data: corresponding to those of (*R*)-**23a**.

(R)-2-[(Benzoylamino)methyl]-3-methylbutanoic Acid (Bz-(*R*)-β^2-HVal-OH; (*R*)-**23b**). According to *GP 3*, (4*S*,2′*R*)-**22b** (19.7 g, 50 mmol) was treated with 30% H_2O_2 soln. (20.5 ml, 200 mmol) and LiOH · H_2O (3.36 g, 80 mmol). Recrystallization from (i-Pr)$_2$O/hexane gave (*R*)-**23b** (9.07 g, 77%). White needles. M.p. 124–126°. $[\alpha]_D^{r.t.} = -11.6$ (*c* = 1.04, $CHCl_3$). IR ($CHCl_3$): 3600–2400(br.), 3452*m*, 2967*s*, 1709*s*, 1655*s*, 1579*m*, 1524*s*, 1488*s*, 1287*m*, 1040*w*, 871*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.01 (*d*, *J* = 6.8, Me); 1.03 (*d*, *J* = 6.7, Me); 2.02–2.14 (*m*, CH); 2.57–2.62 (*m*, CHCO); 3.53–3.60 (*m*, 1 H, CH_2N); 3.77–3.83 (*m*, 1 H, CH_2N); 6.87 (*t*, *J* = 5.8, NH); 7.27–7.39 (*m*, 2 arom. H); 7.44–7.49 (*m*, arom. H); 7.71–7.74 (*m*, 2 arom. H); 9.50 (br., COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.8, 20.3 (Me); 28.7 (CH); 38.7 (CH_2); 51.4, 126.9, 128.6, 131.6 (CH); 134.1, 168.0, 179.3 (C). EI-MS: 235 (< 1, M^+), 189 (3), 135 (8), 134 (9), 105 (100), 77 (50). Anal. calc. for $C_{13}H_{17}NO_3$ (235.28): C 66.36, H 7.28, N 5.95; found: C 66.29, H 7.33, N 5.99.

(S)-2-[(Benzoylamino)methyl]-3-methylbutanoic Acid (Bz-(*S*)-β^2-HVal-OH; (*S*)-**23b**). According to *GP 3*, (4*R*,2′*S*)-**22b** (4.0 g, 10.1 mmol) was treated with 30% H_2O_2 soln. (4.14 ml, 40.3 mmol) and LiOH · H_2O (681 mg, 16 mmol). Recrystallization from Et_2O/hexane gave (*S*)-**23b** (1.88 g, 79%). White needles. R_f 0.64 (AcOEt/hexane/AcOH 5:5:0.5). M.p. 123–125°. $[\alpha]_D^{r.t.} = +14.6$ (*c* = 1.25, $CHCl_3$). Other spectroscopic data: corresponding to those of (*R*)-**23b**.

(R)-2-[(Benzoylamino)methyl]-4-methylpentanoic Acid (Bz-(*R*)-β^2-HLeu-OH; (*R*)-**23c**). According to *GP 3*, (4*S*,2′*R*)-**22c** (4.09 g, 10 mmol) was treated with 30% H_2O_2 soln. (4.10 ml, 40 mmol) an LiOH · H_2O (667 mg, 16 mmol). Recrystallization from (i-Pr)$_2$O gave (*R*)-**23c** (1.89 g, 76%). White needles. M.p. 91–92°. $[\alpha]_D^{r.t.} = -8.6$ (*c* = 1.07, $CHCl_3$). IR ($CHCl_3$): 3600–2400(br.), 3452*m*, 3007*m*, 2962*s*, 1711*s*, 1658*s*, 1580*m*, 1522*s*, 1487*s*, 1288*m*, 1042*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.93 (*d*, *J* = 6.5, Me); 0.93 (*d*, *J* = 6.5, Me); 1.35–1.41 (*m*, CH); 1.60–1.68 (*m*, 1 H, CH_2); 1.70–1.77 (*m*, 1 H, CH_2); 2.81–2.87 (*m*, CHCO); 3.50–3.57 (*m*, 1 H, CH_2N); 3.70–3.76 (*m*, 1 H, CH_2N); 6.85 (*t*, *J* = 5.9, NH); 7.37–7.41 (*m*, 2 arom. H); 7.45–7.50 (*m*, arom. H); 7.71–7.75 (*m*, 2 arom. H); 10.0 (br., COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.4, 22.5 (Me); 25.8 (CH); 38.7, 41.0 (CH_2); 43.1, 127.0, 128.6, 131.6 (CH); 134.1, 168.0, 180.2 (C). EI-MS: 249 (< 1, M^+), 206 (2), 193 (6), 134 (10), 105 (100), 77 (42). Anal. calc. for $C_{14}H_{19}NO_3$ (249.31): C 67.45, H 7.68, N 5.62; found: C 67.59, H 7.62, N 5.71.

(S)-2-[(Benzoylamino)methyl]-4-methylpentanoic Acid (Bz-(*S*)-β^2-HLeu-OH; (*S*)-**23c**). According to *GP 3*, (4*R*,2′*S*)-**22c** (16.78 g, 41.1 mmol) was treated with 30% H_2O_2 soln. (16.75 ml, 163.4 mmol) and LiOH · H_2O (2.84 g, 67.7 mmol). Recrystallization from AcOEt/hexane gave (*S*)-**23c** (5.97 g, 58%). White needles. Spectroscopic data: corresponding to those of (*R*)-**23b** but with opposite sign of optical rotation.

(S)-2-[(Benzoylamino)methyl]-3-phenylpropanoic Acid (Bz-(*S*)-β^2-HPhe-OH; (*S*)-**23d**). According to *GP 3*, (4*R*,2′*S*)-**22d** (3.75 g, 8.5 mmol) was treated with 30% H_2O_2 soln. (3.46 ml, 34 mmol) and LiOH · H_2O (357 mg, 8.5 mmol). Recrystallization from Et_2O/hexane gave (*S*)-**23d** (2.25 g, 93%). White solid. R_f 0.58 (AcOEt/hexane/AcOH 5:5:0.5). M.p. 154°. $[\alpha]_D^{r.t.} = +3.3$ (*c* = 1.09, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3037*w*, 2939*w*, 1715*m*, 1657*m*, 1602*w*, 1579*w*, 1522*m*, 1488*m*, 1289*w*, 1092*w*, 1007*w*. ^{1}H-NMR (200 MHz, CD_3OD): 2.82–3.14 (*m*, PhCH_2, CHCO); 3.57–3.61 (*m*, CH_2N); 6.79 (br., NH); 7.14–7.27 (*m*, 5 arom. H); 7.39–7.56 (*m*, 3 arom. H); 7.75–7.79 (*m*, 2 arom. H); 8.51 (br., NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 35.9, 40.7 (CH_2); 46.6 (CH); 127.1, 127.2, 128.8, 128.9, 129.2, 131.9 (CH); 134.3, 138.1, 138.3, 178.6 (C). FAB-MS: 567 (12, [2*M* + 1]$^+$), 307 (38, [*M* + Na]$^+$), 284 (100, [*M* + 1]$^+$). Anal. calc. for $C_{17}H_{17}NO_3$ (283.33): C 72.07, H 6.05, N 4.94; found: C 72.03, H 6.11, N 4.97.

*Boc-(R)-β²-HAla-(R)-β²-HLeu-OBn (ent-***24**). Compound (*R*)-**13a** (183 mg, 0.90 mmol) in THF (5 ml) was treated with (*R*)-**12c** (417 mg, 1.00 mmol), HOBt (149 mg, 1.10 mmol), NMM (0.28 ml, 2.50 mmol), and EDC (173 mg, 0.90 mmol) according to *GP 8c*. FC (AcOEt/hexane 1:2) yielded *ent*-**24** (343 mg, 91%). White solid. M.p. 113–115°. R_f 0.20 (AcOEt/hexane 1:2). $[\alpha]_D^{r.t.} = -52.6$ (*c* = 1.10, $CHCl_3$). IR ($CHCl_3$): 3450*m*, 3007*m*, 2984*m*, 1708*s*, 1671*m*, 1505*s*, 1455*m*, 1392*m*, 1368*m*, 1171*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, *J* = 6.4, Me); 0.90 (*d*, *J* = 6.4, Me); 1.02 (*d*, *J* = 7.1, Me); 1.27–1.33 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.52–1.67 (*m*, CH_2); 2.34–2.44 (*m*, CHCO); 2.71–2.78 (*m*, CHCO); 3.11–3.30 (*m*, 2 CHN); 3.31–3.36 (*m*, CHN); 3.47–3.53 (*m*, CHN); 5.04 (br., NH); 5.12 (*d*, *J* = 12.3, 1 H, PhCH_2); 5.14 (*d*, *J* = 12.3, 1 H, PhCH_2); 5.95 (br., BocN*H*); 7.31–7.39

(*m*, arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.4, 22.4, 22.5 (Me); 25.8 (CH); 28.4 (Me); 38.7, 40.5 (CH_2); 41.1, 43.3 (CH); 43.5, 66.5 (CH_2); 79.2 (C); 128.3, 128.4, 128.7 (CH); 135.8, 156.2, 175.1, 175.2 (C). FAB-MS: 863 (1, $[2M + Na]^+$), 841 (5, $[2M + 1]^+$), 443 (10, $[M + Na]^+$), 421 (81, $[M + 1]^+$), 365 (44), 321 (100), 257 (21). Anal. calc. for $C_{23}H_{36}N_2O_5$ (420.55): C 65.69, H 8.63, N 6.66; found: C 65.59, H 8.84, N 6.83. **24**: $[\alpha]_D^{r.t.} = +53.3$ ($c = 1.07$, $CHCl_3$).

Boc-(S)-β³-HAla-(S)-β³-HLeu-OBn (**25**). Compound **9c** (3.42 g, 10.2 mmol) was deprotected according to *GP 5a*, the resulting TFA salt dissolved in CH_2Cl_2 and treated with Et_3N (7.1 ml, 50.9 mmol), HOBt (1.97 g, 12.8 mmol), **8a** (2.07 g, 10.2 mmol), and EDC (2.37 g, 12.4 mmol) according to *GP 8a*. FC (AcOEt/pentane 1:3 → AcOEt/pentane 1:1) yielded **25** (3.27 g, 76%). White solid. M.p. 122.5–123.5°. R_f 0.08 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -34.7$ ($c = 0.98$, $CHCl_3$). IR ($CHCl_3$): 3425*m*, 3000*m*, 2950*m*, 2929*m*, 2870*w*, 1725*s*, 1700*s*, 1660*s*, 1495*s*, 1450*m*, 1385*m*, 1350*m*, 1310*s*, 1170*s*, 1100*m*, 1055*m*, 1030*m*, 1000*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.5$, 3 H, Me_2C); 0.89 (*d*, $J = 6.8$, 3 H, Me_2C); 1.17 (*d*, $J = 6.7$, Me); 1.24–1.31 (*m*, CH); 1.41–1.49 (*m*, 1 H, CH_2); 1.43 (*s*, *t*-Bu); 1.51–1.63 (*m*, 1 H, CH_2); 2.25–2.37 (*m*, CH_2CO); 2.50–2.61 (*m*, CH_2CO); 3.87–3.97 (*m*, CHN); 4.30–4.39 (*m*, CHN); $\nu_A = 5.09$, $\nu_B = 5.14$ (*AB*, $J_{AB} = 12.2$, CH_2O); 5.27 (br., BocN*H*); 6.15 (br., NH); 7.30–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.4, 22.0, 22.8 (Me); 24.9 (CH); 28.4 (Me); 39.1, 42.6, 43.0 (CH_2); 44.2 (CH); 66.4 (CH_2); 79.1 (C); 128.3, 128.6 (C); 135.6, 155.3, 170.1, 171.5 (C). FAB-MS: 864 (10), 842 (20), 742 (17), 657 (13), 656 (31), 576 (11), 443 (25, $[M + Na]^+$), 422 (29), 421 (95, $[M + 1]^+$), 420 (12), 419 (11), 365 (25), 322 (26), 321 (100), 236 (11). Anal. calc. for $C_{23}H_{36}N_2O_5$ (420.55): C 65.69, H 8.63, N 6.66; found: C 65.85, H 8.84, N 6.67.

Boc-(S)-β²-HAla-(S)-β³-HLeu-OBn (**26**). Compound **9c** (3.02 g, 9.00 mmol) was deprotected according to *GP 5c* and the resulting HCl salt dissolved in CH_2Cl_2 (18 ml), treated with (*S*)-**13a** (2.07 g, 10.2 mmol), HOBt (1.50 g, 9.78 mmol), NMM (2.80 ml, 25.5 mmol), and EDC (1.86 g, 9.72 mmol) according to *GP 8c*. FC (AcOEt/pentane 1:2 → AcOEt/pentane 1:1) and recrystallization from AcOEt/hexane yielded **26** (2.98 g, 79%). Colorless needles. M.p. 113.0–113.5°. R_f 0.25 (AcOEt/pentane 1:2). $[\alpha]_D^{r.t.} = -23.0$ ($c = 0.85$, $CHCl_3$). IR ($CHCl_3$): 3700*w*, 3445*m*, 3000*m*, 2965*m*, 2945*m*, 1710*s*, 1670*s*, 1505*s*, 1450*s*, 1400*m*, 1370*m*, 1170*s*, 1120*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.5$, Me); 0.89 (*d*, $J = 6.6$, Me); 1.10 (*d*, $J = 7.1$, Me); 1.25–1.32 (*m*, CH); 1.41–1.48 (*m*, 1 H, CH_2); 1.42 (*s*, *t*-Bu); 1.52–1.62 (*m*, 1 H, CH_2); 2.38–2.48 (*m*, CHCO); 2.48–2.62 (*m*, CH_2CO); 3.13–3.28 (*m*, CH_2N); 4.29–4.38 (*m*, CHN); $\nu_A = 5.10$, $\nu_B = 5.15$ (*AB*, $J_{AB} = 12.2$, CH_2O); 5.16 (br., BocN*H*); 5.98 (br., NH); 7.30–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.4, 22.1, 22.8 (Me); 25.1 (CH); 28.4 (Me); 35.3 (CH); 39.2 (CH_2); 41.2 (CH); 43.3, 43.7 (CH_2); 44.3 (CH); 66.5 (CH_2); 79.1 (C); 128.4, 128.4, 128.6 (CH); 135.7, 156.2, 171.6, 174.4 (C). FAB-MS: 841 (18, $[2M + 1]^+$), 443 (22, $[M + Na]^+$), 422 (35), 421 (100, $[M + 1]^+$), 419 (12), 366 (13), 365 (45), 322 (27), 321 (86), 257 (11). Anal. calc. for $C_{23}H_{36}N_2O_5$ (420.55): C 65.69, H 8.63, N 6.66; found: C 65.66, H 8.71, N 6.63.

Boc-(S)-β³-HAla-(S)-β²-HLeu-OBn (**27**). Compound (*S*)-**12c** (1.59 g, 3.89 mmol) was dissolved in CH_2Cl_2 (8 ml), treated with **8a** (0.82 g, 4.04 mmol), HOBt (0.66 g, 4.32 mmol), NMM (1.20 ml, 10.9 mmol), and EDC (0.76 g, 3.99 mmol) according to *GP 8c*. FC (AcOEt/pentane 1:1) and recrystallization from AcOEt/hexane yielded **27** (1.25 g, 77%). White solid. M.p. 113.5–114.0°. R_f 0.45 (AcOEt/pentane 1:1). $[\alpha]_D^{r.t.} = +7.9$ ($c = 0.97$, $CHCl_3$). IR: 3430*m*, 3005*m*, 2955*m*, 2930*m*, 2875*w*, 1705*s*, 1670*s*, 1500*s*, 1455*m*, 1395*m*, 1365*m*, 1170*s*, 1105*m*, 1065*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*d*, $J = 6.4$, 3 H, Me_2C); 0.89 (*d*, $J = 6.5$, 3 H, Me_2C); 1.17 (*d*, $J = 6.7$, Me); 1.23–1.37 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.52–1.68 (*m*, CH_2); 2.20–2.33 (*m*, CH_2CO); 2.71–2.78 (*m*, CHCO); 3.29–3.36 (*m*, 1 H, CH_2N); 3.46–3.52 (*m*, 1 H, CH_2N); 3.86–3.94 (*m*, CHN); $\nu_A = 5.13$, $\nu_B = 5.15$ (*AB*, $J_{AB} = 12.3$, CH_2O); 5.29 (br., BocN*H*); 6.03 (br., NH); 7.31–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.4, 22.4, 22.5 (Me); 25.8 (CH); 28.4 (Me); 38.8, 40.6, 42.5 (CH_2); 43.3, 44.1 (CH); 66.5 (CH_2); 79.2 (C); 128.3, 128.4, 128.7 (CH); 135.8, 155.4, 170.9, 175.2 (C). FAB-MS: 863 (5, $[2M + Na]^+$), 841 (8, $[2M + 1]^+$), 741 (11), 443 (20), 422 (28), 421 (81, $[M + 1]^+$), 366 (11), 365 (43), 322 (43), 321 (100), 319 (19), 257 (16), 236 (14), 231 (12), 170 (17), 134 (10). Anal. calc. for $C_{23}H_{36}N_2O_5$ (420.55): C 65.69, H 8.63, N 6.66; found: C 65.86, H 8.75, N 6.63.

Boc-(2S,3S)-β²,³-HAla(α-Me)-(2S,3S)-β²,³-HLeu(α-Me)-OBn (**28**). Compound **15c** (0.743 g, 2.13 mmol) was Boc-deprotected according to *GP 5a*. The resulting crude TFA salt was coupled with Boc-(2*S*,3*S*)-β²,³-HAla(α-Me)-OH (0.463 g, 2.13 mmol) according to *GP 8b*. FC (AcOEt/pentane 2:7) yielded **28** (0.758 g, 81%). Colorless solid. M.p. 156–158°. R_f 0.29 (AcOEt/pentane 2:7). $[\alpha]_D^{r.t.} = -19.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3419*w*, 3008*m*, 2988*m*, 2931*m*, 2871*w*, 1702*s*, 1660*m*, 1496*s*, 1456*m*, 1392*m*, 1368*m*, 1347*m*, 1170*s*, 1106*w*, 992*w*, 624*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 (*d*, $J = 6.6$, 2 Me); 1.14–1.22 (*m*, 10 H, 3 Me, CH); 1.31–1.38 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.51–1.59 (*m*, CH); 2.23–2.29 (*m*, CHCO); 2.63–2.75 (*m*, CHCO); 3.73–3.74 (*m*, CHN); 4.13–4.20 (*m*, CHN); 5.10 (*d*, $J = 12.2$, 1 H, $PhCH_2$); 5.17 (*d*, $J = 12.2$, 1 H, $PhCH_2$); 5.85 (*d*, $J = 7.5$, NH); 6.25 (*d*, $J = 9.6$, NH); 7.31–7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.26, 15.81, 20.12, 22.15, 22.90 (Me);

24.98 (CH); 28.46 (Me); 42.49 (CH); 43.26 (CH_2); 45.60, 48.85, 48.99 (CH); 66.46 (CH_2); 78.88 (C); 128.24, 128.49, 128.69 (CH); 135.65, 156.00, 175.01, 175.55 (C). EI-MS: 920 (93, $[2M + Na]^+$), 898 (14), 471 (100), 449 (52), 371 (24), 349 (57), 90 (31). Anal. calc. for $C_{25}H_{40}N_2O_5$ (448.60): C 66.91, H 8.99, N 6.24; found: C 66.91, H 8.79, N 6.14.

*Boc-(*R*)-β²-HVal-(*R*)-β²-HAla-(*R*)-β²-HLeu-OBn* (*ent*-**29a**). Compound *ent*-**24** (243 mg, 0.58 mmol) was deprotected according to *GP 5c*, the HCl salt dissolved in THF (5 ml) and treated with (*R*)-**13b** (148 mg, 0.64 mmol), HOBt (95 mg, 0.70 mmol), NMM (0.20 ml, 1.8 mmol), and EDC (123 mg, 0.64 mmol) according to *GP 8c*. FC (Et_2O) yielded *ent*-**29a** (231 mg, 74%). White solid. M.p. 157–159°. R_f 0.25 (Et_2O). $[\alpha]_D^{r.t.} = -69.2$ ($c = 0.95$, $CHCl_3$). IR ($CHCl_3$): 3446*m*, 3007*m*, 2963*s*, 2873*m*, 1707*s*, 1663*s*, 1508*s*, 1456*m*, 1368*m*, 1171*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.89 (*d*, $J = 6.3$, 3 Me); 0.94 (*d*, $J = 6.7$, Me); 1.03 (*d*, $J = 7.0$, Me); 1.23–1.32 (*m*, CH); 1.41 (*s*, *t*-Bu); 1.52–1.65 (*m*, CH_2); 1.80–1.89 (*m*, CH); 2.00–2.13 (*m*, CHCO); 2.33–2.46 (*m*, CHCO); 2.66–2.78 (*m*, CHCO); 3.14–3.57 (*m*, 3 CH_2N); 5.03 (br., BocN*H*); 5.10 (*d*, $J = 12.3$, 1 H, PhCH_2); 5.16 (*d*, $J = 12.3$, 1 H, PhCH_2); 6.03 (br., NH); 6.33 (br., NH); 7.26–7.41 (*m*, arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.6, 20.1, 20.8, 22.3, 22.5 (Me); 25.9 (CH); 28.4 (Me); 28.6 (CH); 38.8 (CH_2); 40.6 (CH); 40.6, 40.8, 42.0 (CH_2); 43.6, 54.0 (CH); 66.6 (CH_2); 79.2 (C); 128.3, 128.4, 128.6 (CH); 135.8, 156.1, 174.4, 175.2 (C). FAB-MS: 1069 (20), 1068 (34, $[2M + 1]^+$), 556 (13, $[M + Na]^+$), 535 (65), 534 (100, M^+), 434 (94), 321 (16). Anal. calc. for $C_{29}H_{47}N_3O_6$ (533.71): C 65.26, H 8.88, N 7.87; found: C 65.24, H 8.70, N 8.03. **29a**: $[\alpha]_D^{r.t.} = +69.6$ ($c = 0.875$, $CHCl_3$).

*Boc-(*R*)-β²-HVal-(*R*)-β²-HAla-(*R*)-β²-HLeu-OH* (*ent*-**29b**). Compound *ent*-**29a** (133 mg, 0.25 mmol) in MeOH (6 ml) and Pd/C (10 mg) were treated according to *GP 6* to yield *ent*-**29b** (110 mg, 99%). Colorless glass. $[\alpha]_D^{r.t.} = -53.5$ ($c = 1.01$, $CHCl_3$). IR ($CHCl_3$): 3446*m*, 3320 (br.), 2965*m*, 1706*s*, 1662*s*, 1514*s*, 1456*m*, 1392*m*, 1368*m*, 1169*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.90 (*d*, $J = 6.8$, Me); 0.93 (*d*, $J = 6.5$, 2 Me), 0.97 (*d*, $J = 6.7$, Me); 1.10 (*d*, $J = 7.0$, Me); 1.23–1.30 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.50–1.68 (*m*, CH_2); 1.74–1.82 (*m*, CH); 2.12–2.17 (*m*, CHCO); 2.55–2.62 (*m*, CHCO); 2.63–2.72 (*m*, CHCO); 3.13–3.41 (*m*, 3 CH_2N); 7.96 (br., NH); 8.09 (br., NH). ^{13}C-NMR (CD_3OD, 100 MHz): 16.1, 20.7, 21.1, 22.5, 23.4 (Me); 27.3 (CH); 28.8 (Me); 29.9 (CH); 40.4 (CH_2); 41.6 (CH); 41.9, 42.6, 43.5 (CH_2); 45.0, 54.9 (CH); 80.1, 158.2, 176.8, 177.7, 178.4 (C). FAB-MS: 910 (12, $[2M + Na]^+$), 888 (32, $[2M + 1]^+$), 466 (28, $[M + Na]^+$), 444 (100, $[M + 1]^+$), 344 (71).

*Boc-(*S*)-β²-HVal-(*S*)-β³-HAla-(*S*)-β²-HLeu-OBn* (**30a**). Compound **27** (0.86 g, 2.05 mmol) was deprotected according to *GP 5c*, the resulting HCl salt dissolved in CH_2Cl_2 (4 ml) and treated with (*S*)-**13b** (0.48 g, 2.08 mmol), HOBt (0.36 g, 2.33 mmol), NMM (0.65 ml, 5.91 mmol), and EDC (0.40 g, 2.11 mmol) according to *GP 8c*. FC (CH_2Cl_2/MeOH 97:3) yielded **30a** (1.00 g, 91%). Colorless glass. M.p. 167–168°. R_f 0.30 (CH_2Cl_2/MeOH 97:3). $[\alpha]_D^{r.t.} = +10.6$ ($c = 0.95$, $CHCl_3$). IR ($CHCl_3$): 3440*m*, 3000*m*, 2940*m*, 2915*m*, 2860*w*, 1710*s*, 1660*s*, 1505*s*, 1470*m*, 1450*m*, 1390*m*, 1365*m*, 1335*w*, 1270*m*, 1170*s*, 1010*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–0.96 (*m*, 6 Me); 1.18 (*d*, $J = 6.7$, Me); 1.26–1.35 (*m*, CH_2CH); 1.41 (*s*, *t*-Bu); 1.53–1.67 (*m*, CH_2); 1.83–1.95 (*m*, 1 H, CHC*H*); 1.98–2.09 (*m*, CHCO); 2.12–2.24 (*m*, CHCO); 2.38–2.41 (*m*, CHCO); 2.70–2.80 (*m*, CHCO); 3.21–3.42 (*m*, 3 H, CH_2N); 3.49–3.55 (*m*, 1 H, CH_2N); 4.23–4.32 (*m*, CHN); $\nu_A = 5.14$, $\nu_B = 5.16$ (*AB*, $J_{AB} = 12.3$, CH_2O); 5.21 (br., BocN*H*); 6.17 (br., NH); 6.74 (br., NH); 7.31–7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.9, 20.0, 20.8, 22.3, 22.4 (Me); 25.8 (CH); 28.4 (Me); 28.5 (CH); 38.7, 40.5, 41.6 (CH_2); 42.4, 43.2, 53.7 (CH); 66.5 (CH_2); 79.0 (C); 128.2, 128.4, 128.6 (CH); 135.7, 156.1, 171.0, 173.5, 175.2 (C). FAB-MS: 1090 (16); 558 (28), 557 (70), 556 (100, $[M + Na]^+$), 536 (15), 535 (57), 534 (83, $[M + 1]^+$), 533 (14, M^+), 532 (11), 460 (10), 457 (13), 456 (44), 436 (18), 435 (65), 434 (93), 432 (15), 321 (15). Anal. calc. for $C_{29}H_{47}N_3O_6$ (533.71): C 65.26, H 8.88, N 7.87; found: C 65.02, H 8.95, N 7.80.

*Boc-(*R*)-β³-HVal-(*S*)-β²-HAla-(*S*)-β³-HLeu-OBn* (**31a**). Compound **26** (1.72 g, 4.09 mmol) was deprotected according to *GP 5c*, dissolved in CH_2Cl_2 (8 ml), and treated with **8b** (0.95 g, 4.10 mmol), HOBt (0.70 g, 4.55 mmol), NMM (1.30 ml, 11.8 mmol), and EDC (0.79 g, 4.55 mmol) according to *GP 8c*. FC (CH_2Cl_2/MeOH 97:3) yielded **31a** (2.00 g, 92%). Colorless glass. M.p. 126–127°. R_f 0.23 (CH_2Cl_2/MeOH 97:3). $[\alpha]_D^{r.t.} = +65.8$ ($c = 0.71$, $CHCl_3$). IR ($CHCl_3$): 3440*w*, 3360*m*, 3310*m*, 3010*m*, 2960*m*, 2935*m*, 2875*w*, 1715*m*, 1695*s*, 1660*s*, 1550*m*, 1510*m*, 1455*w*, 1435*w*, 1395*w*, 1365*m*, 1355*w*, 1305*m*, 1285*m*, 1175*s*, 1125*m*, 1095*m*, 1035*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.85–0.95 (*m*, 4 Me); 1.05 (*d*, $J = 6.8$, Me); 1.25–1.33 (*m*, Me_2CHCH_2); 1.43 (*s*, *t*-Bu); 1.55–1.62 (*m*, Me_2CH); 1.63–1.75 (*m*, CH_2); 2.05–2.12 (*m*, 1 H, CH_2CO); 2.12–2.22 (*m*, MeC*H*); 2.31–2.40 (*m*, 1 H, CH_2CO); 2.48–2.55 (*m*, 1 H, CH_2CO); 2.65–2.71 (*m*, 1 H, CH_2CO); 2.70–2.79 (*m*, CHN); 3.69–3.78 (*m*, CHN); 3.94–4.03 (*m*, CHN); 4.40–4.50 (*m*, CHN); 4.82 (br., BocN*H*); $\nu_A = 5.02$, $\nu_B = 5.15$ (*AB*, $J_{AB} = 12.1$, CH_2O); 7.15–7.24 (br., NH); 7.30–7.44 (*m*, 5 arom. H); 7.49 (br., NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 14.8, 17.8, 19.3, 21.9, 23.1 (Me); 24.9 (CH); 28.3 (Me); 33.4 (CH); 40.9, 41.4 (CH_2); 41.8 (CH); 43.0, 44.0 (CH_2); 45.5, 54.5 (CH); 67.2 (CH_2); 79.6 (C); 128.4, 128.4, 128.5 (CH); 135.6, 156.7, 171.0, 173.2, 174.9 (C). FAB-MS: 1090 (2), 557 (19), 556 (51, $[M + Na]^+$), 535 (34), 534 (74, $[M + 1]^+$), 436 (12), 435 (53), 434 (100), 344 (14), 321 (26), 236 (11), 128 (13). Anal. calc. for $C_{29}H_{47}N_3O_6$ (533.71): C 65.26, H 8.88, N 7.87; found: C 65.05, H 8.92, N 7.74.

Boc-(R)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-OBn (**32a**). Compound **25** (2.25 g, 5.34 mmol) was deprotected according to *GP 5a*, the resulting TFA salt dissolved in CH_2Cl_2, and treated with Et_3N (3.7 ml, 26.6 mmol), HOBt (0.99 g, 6.45 mmol), **8b** (1.27 g, 5.50 mmol), and EDC (1.26 g, 6.57 mmol) according to *GP 8a*. FC ($CHCl_3$/MeOH 97:3) yielded **32a** (1.21 g, 42%). Colorless glass. M.p. 178.5–179.5°. R_f 0.37 ($CHCl_3$/MeOH 97:3). $[\alpha]_D^{r.t.} = -35.0$ ($c = 0.99$, $CHCl_3$). IR ($CHCl_3$): 3445*m*, 3000*m*, 2975*m*, 2930*m*, 2875*w*, 1705*s*, 1660*s*, 1500*s*, 1455*m*, 1390*m*, 1365*m*, 1310*m*, 1170*s*, 1120*m*, 1050*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–0.93 (*m*, 4 Me); 1.18 (*d*, $J = 6.7$, Me); 1.23–1.32 (*m*, 1 H, CH_2CH); 1.43 (*s*, *t*-Bu); 1.34–1.63 (*m*, $CHCH_2$); 1.75–1.83 (*m*, $CHCH$); 2.31–2.41 (*m*, 2 CH_2CO); 2.47–2.62 (*m*, CH_2CO); 3.63–3.75 (*m*, CHN); 4.12–4.25 (*m*, CHN); 4.26–4.40 (*m*, CHN); 5.10 (br., BocN*H*); $\nu_A = 5.09$, $\nu_B = 5.15$ (*AB*, $J_{AB} = 12.2$, CH_2O); 6.37 (br., NH); 6.82 (br., NH); 7.30–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.4, 19.4, 19.9, 22.1, 22.8 (Me); 25.0 (CH); 28.4 (Me); 32.3 (CH); 39.3, 39.5, 42.3, 43.1 (CH_2); 43.2, 44.5, 53.4 (CH); 66.5 (CH_2); 79.2 (C); 128.4, 128.6 (CH); 135.7, 156.1, 170.4, 170.5, 171.6 (C). FAB-MS: 1090 (11), 1069 (21), 1068 (37), 556 (24, $[M + Na]^+$), 536 (11), 535 (51), 534 (97, $[M + 1]^+$), 533 (9, M^+), 435 (46), 434 (100), 321 (12). Anal. calc. for $C_{29}H_{47}N_3O_6$ (533.71): C 65.26, H 8.88, N 7.87; found: C 65.15, H 9.00, N 7.83.

Boc-(2R,3R)-β²,³-HAla(α-Me)-(R)-β²-HVal-(R)-β²-HAla-(R)-β²-HLeu-OBn (**33a**). Compound *ent*-**29b** (267 mg, 0.50 mmol) was deprotected according to *GP 5c*, the HCl salt dissolved in THF (10 ml) and treated with *ent*-**16a** (109 mg, 0.50 mmol), HOBt (81 mg, 0.60 mmol), NMM (0.16 ml, 1.4 mmol), and EDC (96 mg, 0.50 mmol) according to *GP 8c*. FC (AcOEt) yielded **33a** (244 mg, 77%). White solid. M.p. 159–162°. $[\alpha]_D^{r.t.} = -58.3$ ($c = 1.15$, $CHCl_3$). IR ($CHCl_3$): 3443*m*, 3007*m*, 2967*m*, 1702*m*, 1659*s*, 1498*s*, 1368*m*, 1170*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.88–0.90 (*m*, 3 Me); 0.95 (*d*, $J = 6.7$, Me); 1.03 (*d*, $J = 7.1$, Me); 1.13 (*d*, $J = 6.7$, Me); 1.16 (*d*, $J = 7.1$, Me); 1.21–1.32 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.54–1.64 (*m*, CH_2), 1.82–1.89 (*m*, CH); 1.99–2.07 (*m*, CHCO); 2.26–2.31 (*m*, CHCO); 2.40–2.47 (*m*, CHCO); 2.71–2.76 (*m*, CHCO); 3.20–3.29 (*m*, CH_2N); 3.34–3.43 (*m*, CH_2N); 3.47–3.54 (*m*, CH_2N); 3.68–3.78 (*m*, CHN); 5.13 (*AB*, CH_2O); 5.78 (*d*, $J = 9.0$, BocN*H*); 6.10 (*s*, NH); 6.52 (*t*, $J = 6.1$, NH); 6.62 (*s*, NH); 7.32–7.39 (*m*, arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 15.6, 15.7, 19.9, 20.0, 21.0, 22.4, 22.5 (Me); 25.9, 28.4 (CH); 28.5 (Me); 38.8, 39.0 (CH_2); 40.7 (CH); 40.9, 42.2 (CH_2); 43.5, 45.4, 49.0, 53.3 (CH); 66.6 (CH_2); 78.8 (C); 128.3, 128.5, 128.7 (CH); 135.7, 156.0, 174.7, 175.1, 175.2 (C). FAB-MS: 655 (18, $[M + Na]^+$), 633 (60, $[M + 1]^+$), 534 (48), 533 (100).

Boc-(2S,3S)-β²,³-HVal(α-Me)-(2S,3S)-β²,³-HAla(α-Me)-(2S,3S)-β²,³-HLeu(α-Me)-OBn (**34a**). Compound **28** (0.526 g, 1.17 mmol) was Boc-deprotected according to *GP 5a*. The resulting crude TFA salt was coupled with **16b** (0.287 g, 1.17 mmol) according to *GP 8b*. FC (CH_2Cl_2/Et_2O 4:1) yielded **34a**. Colorless powder (0.569 g, 85%). M.p. 179.5–181°. R_f 0.19 (CH_2Cl_2/Et_2O 4:1). $[\alpha]_D^{r.t.} = -32.3$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3412*w*, 3008*m*, 2970*m*, 2936*m*, 2923*m*, 2872*w*, 1703*m*, 1653*m*, 1494*s*, 1456*m*, 1390*w*, 1367*m*, 1174*s*, 1018*w*, 616*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86 (*d*, $J = 3.4$, Me); 0.88 (*d*, $J = 3.6$, Me); 0.93 (*d*, $J = 6.7$, Me); 0.97 (*d*, $J = 6.7$, Me); 1.13 (*d*, $J = 6.7$, Me); 1.15–1.25 (*m*, 3 Me, CH); 1.29–1.38 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.49–1.71 (*m*, 2 CH); 2.22–2.28 (*m*, CHCO); 2.50–2.56 (*m*, CHCO); 2.70–2.76 (*m*, CHCO); 3.28 (*dt*, $J = 9.6$, 3.7, *CH*NHBoc); 3.98–4.06 (*m*, CHN); 4.11–4.18 (*m*, CHN); 5.1 (*d*, $J = 12.2$, 1 H, $PhCH_2$); 5.17 (*d*, $J = 12.2$, 1 H, $PhCH_2$); 6.05 (*d*, $J = 9.9$; NH); 6.33 (*d*, $J = 9.8$, NH); 7.31–7.40 (*m*, NH, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.38, 16.53, 16.66, 19.73, 19.97, 20.30, 22.16, 22.80 (Me); 25.02 (CH); 28.47 (Me); 32.36, 41.31, 42.27 (CH); 43.19 (CH_2); 44.66, 47.26, 49.20, 59.29 (CH); 66.53 (CH_2); 78.24 (C); 128.22, 128.54, 128.71 (CH); 135.58, 156.78, 175.33, 175.47, 175.55 (C). FAB-MS: 1750 (9, $[3M + Na]^+$), 1728 ($3M + 1]^+$), 1174 (100, $[2M + Na]^+$), 1152 (33, $[2M + 1]^+$), 598 (< 1, $[M + Na]^+$). Anal. calc. for $C_{32}H_{53}N_3O_6$ (575.79): C 66.75, H 9.28, N 7.30; found: C 66.66, H 9.21, N 7.23.

Boc-(2S,3S)-β²,³-HVal(α-Me)-(2S,3S)-β²,³-HAla(α-Me)-(2S,3S)-β²,³-HLeu(α-Me)-OH (**34b**). Compound **34a** (0.188 g, 0.327 mmol) was debenzylated in MeOH according to *GP 6*: **34b** (0.16 g, 100%). M.p. 191–193°. R_f 0.38 ($CHCl_3$/MeOH 9:1). $[\alpha]_D^{r.t.} = -9.7$ ($c = 0.37$, CH_3OH). IR ($CHCl_3$): 3411*w*, 2966*s*, 2934*m*, 2874*m*, 1702*s*, 1652*s*, 1496*s*, 1463*m*, 1391*m*, 1368*m*, 1295*m*, 1172*s*, 1100*w*, 1076*w*, 1040*w*, 975*w*, , 889*w*, 864*w*, 652*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85–0.97 (*m*, 4 Me); 1.13–1.36 (*m*, CH, 4 Me); 1.42 (*s*, 6 H, *t*-Bu, rotamer); 1.45 (*s*, 3 H, *t*-Bu, rotamer); 1.58–1.60 (*m*, Me_2CH); 1.69–1.74 (*m*, Me_2CH); 2.31–2.33 (*m*, CHCO); 2.48–2.53 (*m*, CHCO); 2.64–2.67 (*m*, CHCO); 3.37–3.41 (*m*, *CH*NHBoc); 4.03–4.16 (*m*, 2 CHN); 5.84 (*d*, $J = 9.6$, NH); 6.51 (br., $J = 8.5$, NH); 7.37 (*d*, $J = 8.6$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.52, 16.21, 16.36, 18.61, 19.63, 20.25, 22.12, 23.05 (Me); 25.04 (CH); 28.45 (Me), 31.47, 42.14, 42.97 (CH); 43.13 (CH_2); 44.80, 47.50, 49.28, 58.91 (CH); 78.70 (C); 156.78, 175.14, 175.58, 178.2 (C). FAB-MS: 1009 (4, $[2M + K]^+$), 995 (31, $[2M + Na]^+$), 509 (100, $[M + Na]^+$), 487 (20, $[M + 1]^+$), 409 (16), 387 (66), 259 (12), 154 (23), 137 (13).

10. *NMR Spectroscopy of Heptapeptide* **2c**. Sample: 12 mg **2c** dissolved in 0.6 ml of CD_3OH. *1D-NMR-(AMX500)*: ^{1}H-NMR (500 MHz): suppression of the CD_3OH signal by presaturation; 90-K data points, 64 scans, 6.4-s acquisition time. {^{1}H}-BB-decoupled ^{13}C-NMR (125 MHz): 80-K data points, 8000 transients, 1.3-s acqui-

sition time, 45° excitation pulse, 1-s relaxation delay. Processed with 1.0-Hz exponential line broadening. *2D-NMR.* DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection [42] and solvent suppression: *Acquisition:* 2K(t_2) × 512 (t_1) data points. 4 scans per t_1 increment, 0.21-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG [43] (500, 125 MHz, CD_3OH): *Acquisition:* 2K(t_2) × 512 (t_1) data points, 2 scans per t_1 increment. ^{13}C-GARP Decoupling during t_2. 0.21-s acquisition time in t_2. 1.5-s relaxation delay. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HMBC with PFG [44] (500, 125 MHz, CD_3OH): *Acquisition:* solvent suppression by presaturation, no ^{13}C decoupling, otherwise identical to parameters for HSQC. *Processing:* Zero filling and FT to 1K × 1K after multiplication with $\cos^2$ filter in ω_2 and gaussian filter in ω_1; power spectrum in both dimensions. ROESY [45] (500 MHz, CD_3OH): *Acquisition:* A series of 3 ROESY spectra with mixing times of 50, 100, and 150 ms was acquired. Solvent suppression by presaturation, CW-spin lock (3.8 kHz) between trim pulses, 4K(t_2) × 512 (t_1) data points, 32 scans per t_1 increment. 0.422-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to 1K × 1K 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.

11. *NMR Spectroscopy of Hexapeptide* **7c**. Sample: 15 mg **7c** dissolved in 0.6 ml of CD_3OH. *1D-NMR* (AMX500): ^{1}H-NMR (500 MHz): suppression of the $CD_3O\mathit{H}$ signal by presaturation; 90-K data points, 64 scans, 5.6-s acquisition time. {^{1}H}-BB-decoupled ^{13}C-NMR (125 MHz): 80-K data points, 4197 transients, 1.3-s acquisition time, 45° excitation pulse, 1-s relaxation delay. Processed with 1.0 Hz exponential line broadening. *2D-NMR.* DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection and solvent suppression: *Acquisition:* 2K (t_2) × 512 (t_1) data points. 4 scans per t_1 increment, 0.21-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Procssing*: Zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG (500, 125 MHz, CD_3OH): *Acquisition:* 2K (t_2) × 512 (t_1) data points, 2 scans per t_1 increment. ^{13}C-GARP decoupling during t_2. 0.21-s acquisition time in t_2. 1.5-s relaxation delay. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/2$ in ω_2 and with sin filter shifted by $\pi/2$ in ω_1. HMBC with PFG (500, 125 MHz, CD_3OH): *Acquisition:* solvent suppression by presaturation, no ^{13}C decoupling, otherwise identical to parameters for HSQC. *Processing:* Zero filling and FT to 1K × 1K after multiplication with $\cos^2$ filter in ω_2 and gaussian filter in ω_1; power spectrum in both dimensions. ROESY (500 MHz, CD_3OH): *Acquisition:* A series of 3 ROESY spectra with mixing times of 50, 100, and 150 ms was acquired. Solvent suppression by presaturation, CW-spin lock (3.8 kHz) between trim pulses, 4K (t_2) × 768 (t_1) data points, 32 scans per t_1 increment. 0.422-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to 1K × 1K 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.

12. *NMR Spectroscopy of Hexapeptide* **4c**. Sample: 12 mg **4c** dissolved in 0.6 ml of CD_3OH. *1D-NMR* (AMX500): ^{1}H-NMR (500 MHz): suppression of the $CD_3O\mathit{H}$ signal by presaturation; 90-K data points, 128 scans, 5.6-s acquisition time. *2D-NMR.* DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection and solvent suppression: *Acquisition:* 2K (t_2) × 512 (t_1) data points. 2 scans per t_1 increment, 0.21-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and ω_1. ROESY (500 MHz, CD_3OH): *Acquisition:* 2 ROESY spectra with mixing times of 100 and 150 ms were acquired. Solvent suppression by presaturation, CW-spin lock (3.8 kHz) between trim pulses, 2K (t_2) × 768 (t_1) data points, 64 scans per t_1 increment, 0.184-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to 2K × 1K 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.

13. *NMR Structure Determination.* Energy minimization, molecular dynamics (MD) simulations, and simulated annealing (SA) [32] of **2c** and **7c** were performed with the AMBER* [33] force field implemented in the BatchMin (MacroModel) version 5.0 [46] on *Silicon Graphics Indy* and O_2 *(R 10000)* workstations under Irix 5.3 and Irix 6.3. The calculation of hexapeptide **4c** was done using *X-PLOR 3.851* [47] on a *Silicon Graphics* O_2 *(R 10000)* workstation. Visualization and manipulation were carried out using MacroModel 5.0, Visual Molecular Dynamics (VMD) [48], MolMol [35], and Raster3D [36] on *Silicon Graphics Indy* and O_2 *(R 10000)* workstations. The MacroModel default parameters were used throughout the simulations of **2c** and **7c** except otherwise noted. All H-atoms were explicitly included in the calculations, covalent bonds to the H-atoms being kept fixed by the SHAKE algorithm [49]. Energy minimization (EM) was performed using the PR conjugate gradient (PRCG) method. All MD simulations were coupled to a thermal bath with a bath constant of 0.2 ps [50].

During SA, the force constant for the distance restraints was 100 kJ/Å^2 and for dihedral angle restraints 1 kcal/mol · rad^2 with 2 for the exponent of the restraining function [47], respectively. SA was carried out *in vacuo* except otherwise noted.

13.1. *Structure of Heptapeptide* **2c**. A model of **2c** based on the average NMR structure of a β-heptapeptide examined previously was generated with neutral N- and C-termini and subjected to a 500-steps EM. After setting the initial temp. to 700 K, a 3-ps equilibration run at 700 K was performed. 50 starting structures were generated by unrestrained molecular dynamics at 700 K (time step 1.0 fs) during a 20-ps run.

The NOEs derived from ROESY experiments described above were classified in three categories according their estimated cross-peak volume in the contour plot: strong, medium, and weak with 3.0 Å, 3.5 Å, and 4.5 Å, respectively, as upper bound distance restraints, and with their respective *van der Waals* radii as lower bound distance restraints.

Each structure was then subjected to restrained SA from 700 to 1 K in 15 ps (time step 1.0 fs) with 57 NOE distance restraints. The twenty structures lowest in energy were selected, heated to 300 K, and then equilibrated during 2 ps with no restraints. A second SA was then performed with 57 NOE distance restraints and 13 additional torsion angle restraints derived from the ^3J coupling constants *via* modified *Karplus* equations (*Eqn. 1*), [51] from 700 K to 1 K in 7 ps. After a 500 steps EM, the ten structures of lowest energy and no restraint violation converged well and gave the final structural bundle (RMSD 1.37 Å ± 0.71 Å for all heavy atoms).

$$^3J(\mathrm{H,H}) = A\cos^2\theta + B\cos\theta + C \qquad (1)$$

with $A = 6.4$ Hz, $B = -1.4$ Hz, $C = 1.9$ Hz for ^3J(HN,HC) [52]

and $A = 9.5$ Hz, $B = -1.6$ Hz, $C = 1.8$ Hz for ^3J(HC,HC) [53]

13.2. *Structure of Hexapeptide* **7c**. A model from a 3_1 helix was generated and subjected to a 500 EM. The initial temp. was set to 700 K, and a 2-ps equilibration run at 700 K was performed (time step 1.0 fs). 50 starting structures were generated by unrestrained molecular dynamics at 500 K (time step 1.5 fs) during a 5-ps run. Each of the 50 structures was then equilibrated at 500 K and then used in SA from 500 to 1 K in 15 ps with 25 NOE restraints classified according the criteria discussed above and 13 torsion angle restraints derived from the ^3J coupling constants *via Eqn. 1*. The GB/SA solvation model for H_2O was selected with following cut-off distances: 7 Å for *van der Waals*, 1 Å for *Coulomb* electrostatic, 1 Å for H-bonding, and 1 Å for charge/multipole electrostatic forces. After 500-steps EM, the six structures lowest in energy with no restraint violations were selected and gave the final structural bundle.

13.3. *Structure of Hexapeptide* **4d** *in* CD_3OH. The X-PLOR files parallhdg.pro and topallhdg.pro [47], containing the parameter and topology data, were modified *by hand* for β-amino acids. A molecular structure was generated by X-PLOR (*generate.inp*) using an initial model (MacroModel 5.0) and subjected to 30-ps unrestrained SA to give a reasonable starting structure.

The diastereotopic CH_2 protons of residues 2, 3, and 4 were assigned such that the larger ^3J values (11.0–12.0 Hz; see *Table 14*) result from coupling between H_{Re}–C(α) and H–C(β) for residues 2 and 4, and between H_{Re}–C(β) and H–C(α) for residue 3. 34 NOEs were ordered according to their cross-peak volume in the contour plot of the 100-ms ROESY in three categories: strong, medium, and weak with 3.0 Å, 3.5 Å and 4.5 Å, respectively, as upper bound distance restraints and their *van der Waals* radii as the lower bound distance restraints. 11 coupling constants were then transformed in dihedral angle restraints using *Eqn. 1* with a tolerance of 30°. The *ab initio* protocol *sa.inp* [54] of X-PLOR 3.851 was used to generate 84 structures. Initial time: 1500 K, 8000 high steps, 6000 cooling steps, 3-fs time step, NOE scaling 200, all other parameters were left unchanged. The resulting structures were analyzed using the protocol *accept.inp* with the following acceptance criteria: no NOE violations > 0.3 Å, no dihedral angle restraints violations > 5°. 30 structures fulfilled this test and were clustered in two different conformers. One is lower in energy (306 ± 2 kcal/mol; RMSD 0.77 ± 0.24 Å for all heavy atoms) one higher (333 ± 15 kcal/mol; RMSD 0.91 ± 0.23 Å).

14. *MD Simulation of Hexapeptide* **4c**. A 10-ns MD simulation (time step 2 fs) at 50 K was performed using the GB/SA solvation model for water. Following cut-off *radii* were selected: 8 Å for *van der Waals*, 20 Å for *Coulomb* electrostatic, 4 Å for H-bonding, and 10 Å for charge/multipole electrostatic forces. All other parameters (default values) were left unchanged.

15. *Exchange Kinetics of Amide Protons* (^{1}H-NMR, 200 or 300 MHz, 24.5°). The samples were either evaporated to dryness under h.v. or lyophylized (H_2O or dioxane) before dissolving it in CD_3OD. The concentrations were in the range of 10–16 mg of peptide in 0.7 ml of CD_3OD. ^{1}H-NMR Spectra were taken at different times, covering 2 to 3 times the half-life of the corresponding amide proton. The intensity of each NH signal was

normalized relative to the corresponding value for a non-exchangeable peak for each data set. First-order rate constants, k, were calculated from the slope of the plot of $\ln[I(NH_{exchangeable})/I(H_{nonexchangeable})]$ *vs.* time.

REFERENCES

[1] D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1997**, *80*, 2033.
[2] T. Hintermann, D. Seebach, *Synlett* **1997**, 437.
[3] Feature article: D. Seebach, J. L. Matthews, *J. Chem. Soc., Chem. Commun.* **1997**, *21*, 2015.
[4] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.
[5] D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 2043.
[6] X. Daura, W. F. van Gunsteren, D. Rigo, B. Jaun, D. Seebach, *Chem. Eur. J.* **1997**, *3*, 1410.
[7] J. L. Matthews, M. Overhand, F. N. M. Kühnle, P. E. Ciceri, D. Seebach, *Liebigs. Ann./Recueil* **1997**, 1371.
[8] D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, *80*, 173.
[9] a) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071; b) D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi Jr, S. H. Gellman, *Nature (London)* **1997**, *387*, 381.
[10] Krauthäuser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719.
[11] T. Hintermann, D. Seebach, *Chimia* **1997**, *51*, 244.
[12] a) G. Quinkert, E. Egert, C. Griesinger, 'Aspects of Organic Chemistry – Structure', VHCA, Basel and VCH, Weinheim, 1996; b) B. W. Gung, Z. Zhu, *J. Org. Chem.* **1997**, *62*, 6100.
[13] B. Penke, J. Czombos, L. Baláspiri, J. Petres, K. Kovács, *Helv. Chim. Acta* **1970**, *53*, 1057.
[14] J. Podlech, D. Seebach, *Liebigs Ann.* **1995**, 1217.
[15] E. Juaristi, 'Enantioselective Synthesis of β-Amino Acids', Wiley-VCH, New York, 1997.
[16] J. L. Matthews, C. Braun, C. Guibourdenche, M. Overhand, D. Seebach, in [15], pp. 105–126.
[17] E. Juaristi, O. García-Barradas, in [15], pp. 139–149 and ref. cit. therein.
[18] D. Seebach, D. Wasmuth, *Angew. Chem.* **1981**, *11*, 1007; *ibid., Int. Ed. Engl.* **1981**, *20*, 971.
[19] D. Seebach, H. Estermann, *Tetrahedron Lett.* **1987**, *28*, 3103; H. Estermann, D. Seebach, *Helv. Chim. Acta* **1987**, *71*, 1824; H. Estermann, Dissertation ETH-Zürich, No. 8538, 1988.
[20] D. Seebach, A. K. Beck, A. Studer, in 'Modern Synthetic Methods 1995', Eds. B. Ernst and C. Leumann, VHCA, Basel, and VCH, Weinheim, 1995, Vol. 7, pp. 1–178.
[21] D. Seebach, S. Abele, unpublished results, ETH-Zürich, 1997.
[22] D. Seebach, B. Weidmann, L. Widler, in 'Modern Synthetic Methods 1983', Ed. R. Scheffold, Salle + Sauerländer, Aarau, and J. Wiley and Sons, New York, 1983, Vol. 3, pp. 217–353, and ref. cit. therein.
[23] G. Shapiro, M. Marzi, *J. Org. Chem.* **1997**, *62*, 7096.
[24] D. A. Evans, F. Urpi, T. C. Somers, S. C. Clark, M. T. Bilodeau, *J. Am. Chem. Soc.* **1990**, *112*, 8215.
[25] J. R. Gage, D. A. Evans, *Org. Synth.*, Coll. Vol. VIII, 339.
[26] G. Guichard, D. Seebach, *Chimia* **1997**, *51*, 315; G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *52*, 187.
[27] R. W. Woody, in 'The Peptides: Conformation in Biology and Drug Design', Ed. V. J. Hruby, Academic Press, Orlando, 1985, Vol. 7, p. 15; R. W. Woody, in 'Circular Dichroism, Principles and Applications', Eds. K. Nakanishi, N. Berova, and R. W. Woody, 1994, Chapt. 17, p. 473.
[28] K. A. Bode, J. Applequist, *Macromolecules* **1997**, *30*, 2144.
[29] W. Qiwen, A. D. Kline, K. Wüthrich, *Biochemistry* **1987**, *26*, 6488.
[30] C. A. Rohl, J. M. Scholtz, E. J. York, J. M. Stewart, R. L. Baldwin, *Biochemistry* **1992**, *31*, 1263.
[31] K. Wüthrich, 'NMR of Proteins and Nucleic Acids', Wiley, New York, 1986.
[32] S. Kirkpatrick, C. D. Gelatti, Jr., M. P. Vecchi, *Science (Washington)* **1983**, *220*, 671.
[33] a) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta, Jr., P. Weiner, *J. Am. Chem. Soc.* **1984**, *106*, 765; b) S. J. Weiner, P. A. Kollman, D. T. Nguyen, D. A. Case, *J. Comp. Chem.* **1986**, *7*, 230; c) D. Q. McDonald, W. C. Still, *Tetrahedron Lett.* **1992**, *33*, 7743; d) D. Q. McDonald, W. C. Still, *ibid.* **1992**, *33*, 7747.
[34] W. F. van Gunsteren, H. J. C. Berendsen, *Angew. Chem.* **1990**, *102*, 1020; *ibid., Int. Ed. Engl.* **1990**, *29*, 992.

[35] R. Koradi, M. Billeter, K. Wüthrich, *J. Mol. Graphics* **1996**, *14*, 51.
[36] a) E. A. Merrit, M. E. P. Murphy, *J. Appl. Crystallogr.* **1994**, *24*, 946; b) D. J. Bacon, W. F. A. Anderson, *J. Mol. Graphics* **1988**, *6*, 219.
[37] X. Daura, B. Jaun, D. Seebach, W. F. van Gunsteren, A. E. Mark, *J. Mol. Biol.*, submitted.
[38] a) B. W. Gung, Z. Zhu, B. Everingham, *J. Org. Chem.* **1997**, *62*, 3436; b) G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc* **1994**, *116*, 1054.
[39] A. Banerjee, P. Balaram, *Curr. Science* **1997**, *73*, 1067.
[40] P. Lombardi, *Chem. Ind. (London)* **1990**, Nov. 5, 708; S. Moss, *ibid.* **1994**, Feb. 21, 122.
[41] E. Juaristi, D. Quintana, M. Balderas, E. García-Pérez, *Tetrahedron: Asymmetry* **1996**, *7*, 2233.
[42] A. L. Davis, E. D. Laue, J. Keeler, D. Moskau, J. Lohman, *J. Magn. Reson.* **1991**, *94*, 637.
[43] A. L. Davis, J. Keeler, E. D. Laue, D. Moskau, *J. Magn. Reson.* **1992**, *98*, 207.
[44] W. Willker, D. Leibfritz, U. Kerssebaum, W. Bermel, *J. Magn. Reson.* **1993**, *31*, 287.
[45] C. Griesinger, R. R. Ernst, *J. Magn. Reson.* **1987**, *75*, 261.
[46] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comp. Chem.* **1990**, *11*, 440.
[47] A. T. Brünger, in 'X-PLOR Manual V3.0', Eds. Y. University, New Haven, 1992.
[48] W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graphics* **1996**, *14*, 33.
[49] a) J. P. Ryckaert, G. Cicotti, H. J. C. Berendsen, *J. Comp. Phys.* **1977**, *23*, 327; b) W. F. van Gunsteren, M. Karplus, *Macromolecules* **1982**, *15*, 1528.
[50] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola, J. R. Haak, *J. Chem. Phys.* **1984**, *8*, 3684.
[51] M. Karplus, *J. Chem. Phys.* **1959**, *30*, 11.
[52] A. Pardi, M. Billeter, K. Wüthrich, *J. Mol. Biol.* **1984**, *180*, 741.
[53] A. de Marco, M. Llinas, K. Wüthrich, *Biopolymers* **1978**, *17*, 617.
[54] a) M. Nilges, G. M. Clore, A. M. Gronenborn, *FEBS Lett.* **1988**, *239*, 129; b) M. Nilges, J. Kuszewski, A. T. Brünger, in 'Computational Aspects of the Study of Macromolecules by NMR', Eds. J. C. Hoch, F. M. Poulsen, C. Redfield, Plenum Press, New York, 1991.

Received February 19, 1998