80. β-Peptides: Synthesis by *Arndt-Eistert* Homologation with Concomitant Peptide Coupling. Structure Determination by NMR and CD Spectroscopy and by X-Ray Crystallography. Helical Secondary Structure of a β-Hexapeptide in Solution and Its Stability towards Pepsin

by **Dieter Seebach***, **Mark Overhand**[1]), **Florian N. M. Kühnle**[2]), and **Bruno Martinoni**

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

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

Präklinische Forschung, *Sandoz Pharma AG*, CH–4002 Basel

(1.III.96)

The β-hexapeptide (H-β-HVal-β-HAla-β-HLeu)$_2$-OH (**2**) was prepared from the component L-β-amino acids by conventional peptide synthesis, including fragment coupling. A cyclo-β-tri- and a cyclo-β-hexapeptide were also prepared. The β-amino acids were obtained from α-amino acids by *Arndt-Eistert* homologation. All reactions leading to the β-peptides occur smoothly and in high yields. The β-peptides were characterized by their CD and NMR spectra (COSY, ROESY, TOCSY, and NOE-restricted modelling), and by an X-ray crystal-structure analysis. β-Sheet-type structures (in the solid state) and a compact, left-handed or (*M*) 3_1 helix of 5-Å pitch (in solution) were discovered. Comparison with the analogous secondary structures of α-peptides shows fundamental differences, the most surprising one at this point being the greater stability of β-peptide helices. There are structural relationships of β-peptides with oligomers of β-hydroxyalkanoic acids, and dissimilarities between the two classes of compounds are a demonstration of the power of H-bonding. The β-hexapeptide **2** is stable to cleavage by pepsin at pH 2 in H_2O for at least 60 h at 37°, while the corresponding α-peptide H-(Val-Ala-Leu)$_2$-OH is cleaved instantaneously under these conditions. The implication of the described results are discussed.

Peptides and proteins are molecules central to life on our planet. When proteinogenic or ribosomal, they consist of α-amino acids linked together by amide bonds. We have recently embarked on a project aimed at the synthesis of oligomers from β-amino acids (β-peptides), in order to be able to compare the structures and the properties of these unnatural peptide analogs with those of the natural products. Another point which attracted our interest in the β-oligopeptides is their resemblance with poly(β-hydroxyalkanoates) (PHA), an ubiquitous class of biopolymers which have been the subject of research in our group for many years [1].

1. Introduction. – β-Amino acids are much less frequent in nature than α-amino acids [2]. Not only are certain β-amino acids as monomers biologically active [3], they have also been found incorporated into naturally occurring peptides with important pharmacologi-

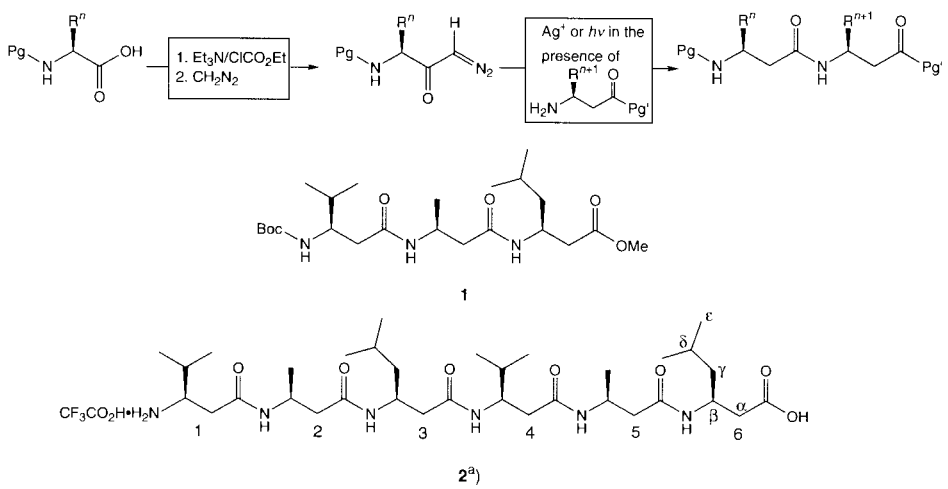
[1]) Postdoctoral research fellow, ETH-Zürich, 1995.

[2]) Part of the projected dissertation of *F. N. M. K.*

cal properties [4]. Because of the additional α-methylene group, β-amino acids represent a class of conformationally more flexible compounds as compared to the α-amino analogs. In contrast, modern amino-acid chemistry aims to develop conformationally restricted amino acids which, after incorporation into peptides, may enhance the biological activity by decreasing the degree of freedom of the peptide to undergo rotation around certain single bonds [5]. However, some peptides in which an α-amino-acid residue was replaced by a β-amino-acid residue at a specific position in the peptide sequence have shown increased stability against certain peptidases with retention or improvement of the biological activity [2] [6]. An important class of β-amino-acid derivatives are the antibiotic β-lactams [7].

The synthesis of β-amino acids has been extensively studied, and two reviews have appeared recently [8]. Ideally, a route is chosen, from the large array of methods available for β-amino-acid synthesis, in which the β-amino acid is produced enantioselectively from a readily available starting material. α-Amino acids are cheap, enantiomerically pure, commercially available compounds which have frequently served as starting materials for the synthesis of β-amino acids [8]. This conversion can be achieved in only two steps using the *Arndt-Eistert* homologation of *N*-protected α-amino acids [9], a method which is especially attractive as the reactive intermediate arising from the *Wolff* rearrangement of a diazo ketone can be trapped with the amine functionality of an amino-acid derivative [10]. By applying this strategy, a β-amino-acid derivative is produced with concomitant peptide coupling. Chain elongation at the N-terminus, by *N*-deprotection and repetition of the *Arndt-Eistert* homologation with another α-amino-acid-derived diazo ketone, produces a peptide which contains a sequence of β-amino acids (*Scheme 1*).

Scheme 1. *The Sequential* Arndt-Eistert *Homologation with Concomitant Amide Formation and Target Molecules* **1** *and* **2**. Pg, Pg′ = Protecting group or adjacent β-amino-acid moiety.



1

2[a])

[a]) The notations α, β, *etc.* are used in the NMR part to assign the proton signals. The numbers *1*, *2*, *etc.* refer to the residue numbering.

Here, we report on the synthesis of the tripeptide Boc-β-HVal-β-HAla-β-HLeu-OMe[3]) (**1**) which contains exclusively β-amino acids, using the sequential *Arndt-Eistert* homologation with concomitant peptide-bond formation, as mentioned above (*Scheme 1*). The α-peptide analog of this tripeptide (*i.e.*, Val-Ala-Leu) has been previously used as a building block to study the influence on peptide conformation in sequences containing α,α-disubstituted amino acids[4]) [11]. Thus, the tripeptide Boc-β-HVal-β-HAla-β-HLeu-OMe (**1**) was chosen to be used in fragment couplings to produce, *e.g.*, the hexapeptide $CF_3CO_2H \cdot$ H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**2**) containing six β-amino acids (*Scheme 1*)[5]). This would enable us to study the conformation of the β-peptides **1** and **2** and to compare their structures not only with those of the α-peptide analogs but also with those of the oligo(3-hydroxyalkanoic acid) derivatives (the β-peptides are the amide analogs of the oligo(3-hydroxyalkanoates) [1]).

Until now, there are only few and partially controversial reports on the structure of β-peptides and of β-amino-acid polymers. *Dado* and *Gellman* have studied achiral β- and γ-dipeptides by IR spectroscopy to predict the folding of polypeptides with unnatural backbones[6]) [15]. They suggest that 'polymers composed of β-amino acids may adopt compact and specific folding patterns, because nearest neighbor hydrogen bond formation is not a favorable process'. The structure of several polydisperse poly(β-amino acids), the so-called nylon-3 derivatives, have been extensively studied in both the solid phase and in solution [16]. Poly[(*S*)-β-aminobutanoic acid], poly(β-aspartic acid), and poly(β-alanine) are thought to have a β-sheet-type conformation [16a–i]. *Yuki et al.* initially concluded from IR, CD, powder X-ray, and NMR investigations that poly-(α-isobutyl-L-aspartate) also forms a β-sheet-type secondary structure both in the solid phase and in solution [16j]. However, more recently it was derived from fiber X-ray scattering that the same polymer adopts helical structures[7]) [16k,l].

Drey and coworkers have synthesized certain monodisperse small linear and cyclic peptides consisting of β-amino acids, including a hexapeptide which is constructed from six identical 3-amino-2,2-dimethylpropanoic acid (β-aminopivalic acid) units [17a–f]. However, no structure has been reported of any of these interesting compounds[8]). Furthermore, certain short-chain β-peptides have been shown to inhibit blood-platelet aggregation and cell-cell adhesion processes [18].

---

[3]) The notation β-HXaa for a homolog of the α-amino acid Xaa was introduced by *Ondetti* and coworkers [6a] and has been used by others [6f]. Unfortunately, the CIP priority sequence is reversed when we go from valine to β-homovaline, so that (*S*)- or L-valine is converted to (*R*)- or L-β-homovaline upon homologation with retention of configuration! β-HXaa implies L-configuration.

[4]) Amino-acid sequences containing apolar side chains permit the investigation of peptide conformation in organic solvents [12]. In organic solvents, the not fully understood so-called hydrophobic effects on peptide folding, which predominate in aqueous solution, are eliminated [11a] [13].

[5]) The X-ray structure of a 14-mer containing the α-peptide analog of this sequence (*i.e.*, Boc-Val-Ala-Leu-Aib(-Val-Ala-Leu)$_2$-Aib-Val-Ala-Leu-OMe) has been reported [14].

[6]) These authors used the names β- and γ-peptides for oligomers consisting of β- and γ-amino acids.

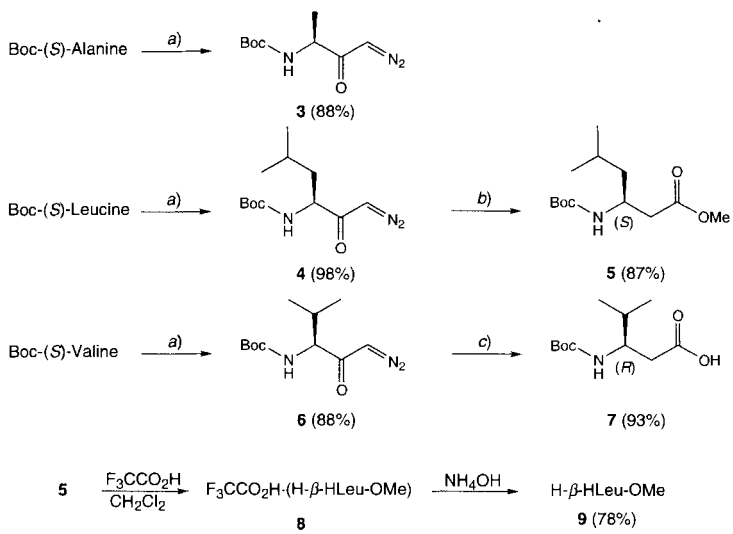
[7]) *Subirana* and coworkers derived mainly from X-ray fiber diffraction that poly(α-isobutyl-L-aspartate) can exist in two types of helical structures, one of which is also stable in a certain solvent system [16k,l]. Both a left-(*M*) and a right-handed (*P*) helix model fit their data, the authors favor the (*M*)-configuration. They argue that because of the additional CH_2 group in the backbone of poly(α-isobutyl-L-aspartate), a 'greater conformational versatility is created when compared with the polypeptide backbone found in proteins'. No comment is given about the completely different (β-sheet-type) structure proposed by *Yuki et al.* [16j].

[8]) Monodisperse β-oligopeptides consisting of L-aspartic acid have also been reported without structural determinations [17g].

Here, we report for the first time on the solution structure of a small uniform peptide consisting of β-amino acids (*i.e.*, β-hexapeptide **2**).

2. Preparative Results. – The commercially available *N*-Boc-protected amino acids (Boc = (*tert*-butoxy)carbonyl) were converted to the mixed anhydrides with Et_3N/$ClCO_2Et$ and subsequently allowed to react with diazomethane, according to the literature procedure [9c, d]. The diazo ketones **3**, **4**, and **6** were obtained in good yields after silica-gel chromatography or crystallization[9]) (*Scheme 2*). The X-ray structure of diazo ketone

Scheme 2. *The* Arndt-Eistert *Procedure: Preparation of the Diazo Ketones* **3**, **4**, *and* **6**, *and* Wolff *Rearrangement to the Homologated Methyl Ester* **5** *and the Carboxylic Acid* **7**



a) 1. Et_3N/$ClCO_2Et$ (−15°); 2. CH_2N_2 (−5° → r.t.). *b*) Cat. $PhCO_2Ag$ in Et_3N/MeOH. *c*) Cat. CF_3CO_2Ag in Et_3N and THF/H_2O 9:1.

4, which crystallized from ethyl acetate/hexane, is depicted in *Fig. 1*. The diazo ketone **4** was decomposed in MeOH as solvent, in the presence of a catalytic amount of silver benzoate[10]) added as a solution in Et_3N [9c]: *N*-Boc-Protected β-homo leucine methyl ester **5** (Boc-β-HLeu-OMe) was obtained in 87% yield[11]). Diazo ketone **6** was decomposed in THF as solvent which contained 10% of H_2O, in the presence of a catalytic amount of silver trifluoroacetate[12]) added as a homogeneous solution in Et_3N. The

[9]) Diazo ketones produced *via* this method have been shown to be enantiomerically pure, and they can be stored for prolonged periods of time at −25° without decomposition [10d–f].

[10]) Silver-based catalysts have frequently been used to induce the *Wolff* rearrangement of diazo ketones [9f] [19].

[11]) For detailed discussions of the mechanisms of the *Wolff* rearrangement, see [19] [20].

[12]) *Newman* and *Beal* reported on a reaction in which CF_3CO_2Ag dissolved in benzene was added to a solution containing a diazo ketone [9c]. The *Wolff* rearrangement was started upon addition of Et_3N. However, to our knowledge, the homogeneous solution of a catalytic amount of CF_3CO_2Ag dissolved in Et_3N has not been reported previously to induce a *Wolff* rearrangement.

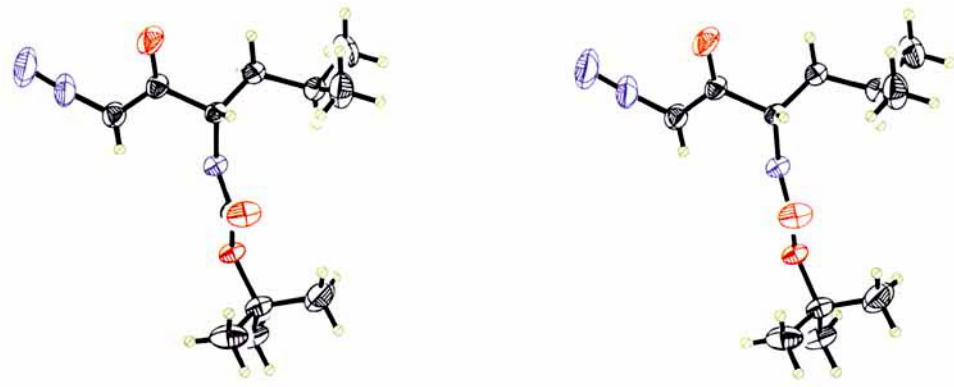
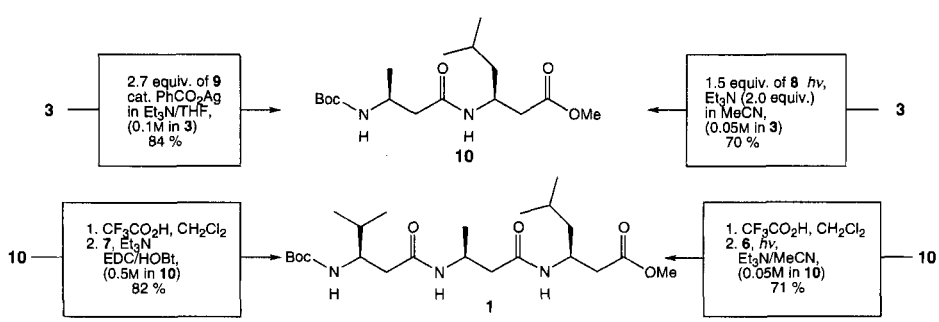


Fig. 1. *Stereoscopic ORTEP representation of diazo ketone* **4**. O-Atoms in red, N-atoms in blue, C-atoms in black, and H-atoms in green. The thermal elipsoids are drawn to the 50% probability level.

desired Boc-protected β-homovaline **7** (Boc-β-HVal-OH) was obtained in a yield of 93%[13]). Compound **5** was Boc-deprotected with the aid of CF_3CO_2H (→ **8**) and converted to the amino ester **9**.

Recently, two different groups have reported on the trapping of the reactive intermediate, which arises from the *Wolff* rearrangement of an amino-acid-derived diazo ketone, with the amino group of a β-amino-acid derivative [10c,d]. By applying this strategy, a homologated amino-acid residue is formed with concomitant peptide-bond formation. A comparison of the two methods is outlined in *Scheme 3*. Thus, *Wolff* rearrangement of diazo ketone **3** with 2.7 equiv. of β-amino ester **9** as the nucleophile in the presence of a catalytic amount of silver benzoate dissolved in Et_3N gave the desired dipeptide **10** in

Scheme 3. *Synthesis of the β-Peptides* **1** *and* **10**: *Comparison of the* Ag^+*-Catalyzed and the Photochemically Induced* Wolff *rearrangement*. EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole.



[13]) The advantage of the use of CF_3CO_2Ag is that the CF_3CO_2H formed on workup is volatile and H_2O-soluble allowing an easy separation from the homologated carboxylic acid. Use of a solution of silver benzoate in Et_3N in the reaction of diazo ketone **6** gave the homologated carboxylic acid **7** from which it was difficult to remove the remaining benzoic acid by either crystallization or chromatography.

84% yield. Alternatively, irradiation of **3** in the presence of 1.5 equiv. of the trifluoroacetate salt **8** and 2.0 equiv. of Et_3N with light from a low-pressure Hg lamp gave **10** in 70% yield. Although the silver-catalyzed transformation of **3** produced the dipeptide **10** in higher yield, more than 2.0 equiv. of the nucleophile **9** are required[14]). Moreover, this reaction only occurred with a non-protonated amino group as nucleophile (see **9**) and not with the corresponding hydrochloride or trifluoroacetate (see **8**). The photochemical conditions gave a lower yield of dipeptide **10**, but only 1.5 equiv. of the salt **8** were required.

The overall yield of the trapping reaction and the number of equiv. of the β-amino-acid nucleophile necessary to produce the desired β-peptide in good yield are both important factors in determining which of the two methods to choose for a homologation reaction with simultaneous amide formation[15]). Guided by these considerations, it was decided to use the photochemical reaction in a small-scale snythesis of tripeptide **1** from dipeptide **10**, thus avoiding the use of an excessive number of equiv. of the deprotected form of **10**: the Boc group in **10** was removed, and 1.5 equiv. of the obtained trifluoroacetate were allowed to react under irradiation with diazo ketone **6** to produce trimer **1** in 71% yield (*Scheme 3*). Alternatively, a standard peptide coupling between compound **7** and the trifluoroacetate obtained from **10** was conducted in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxy-1*H*-benzotriazole (HOBt) [21]. In a previous synthesis of peptides consisting of β-amino acids, the use of carbodiimide as activating reagent proved troublesome in certain cases [17]. In our case, compound **1** was obtained in 82% yield. The standard peptide coupling has the advantage that a large-scale reaction can be easily performed, and that both reaction partners are used in stoichiometric amounts. The synthesis of peptide **1** *via* the *Wolff* rearrangement is a more elegant and shorter route towards this compound than a standard peptide coupling, because amide formation and homologation are combined in one step. The trimer **1** crystallized from MeOH, and its X-ray crystal structure is described in *Sect. 3*.

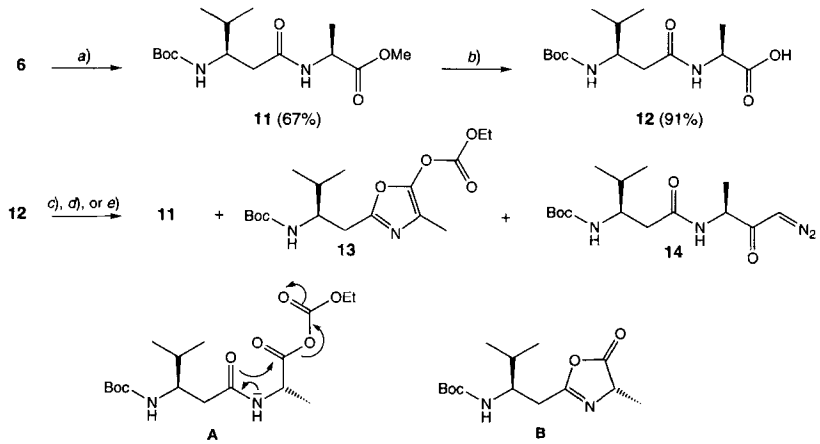
The route towards tripeptide **1** as depicted in *Scheme 3* represents a chain elongation at the N-terminus of the last β-amino acid (*i.e.*, Boc-β-HLeu-OMe (**5**)) of the sequence. It can be envisioned that tripeptide **1** could also be synthesized *via* sequential *Arndt-Eistert* homologation with concomitant peptide coupling starting with the first amino acid of the sequence and elongating at the C-terminus. The result of an attempt of such an approach is detailed in *Scheme 4*. The valine-derived diazo ketone **6** was photochemically decomposed and the reactive intermediate trapped with the commercially available HCl · H-Ala-OMe. The dipeptide analog **11** was obtained and saponified with aqueous NaOH solution to give the carboxylic acid **12**. Activation of the carboxylic acid with $Et_3N/ClCO_2Et$ at −15° and subsequent addition of a solution of CH_2N_2 in Et_2O, following the procedure outlined in *Scheme 2*, did not lead to the desired diazo ketone **14**. Instead, a mixture of

---

[14]) It was shown previously that the product yield under these conditions depends on the number of equiv. of the applied nucleophile [10d].

[15]) Another important factor is the batch size and the dilution of the reactands: In our laboratory, the photochemical reaction to produce tripeptide **1** was performed with a low-pressure Hg lamp yielding at maximum slightly more than 1 g of tripeptide. Because a dilute solution (0.05M) is desirable in the photochemical reaction, a larger quartz vessel is required for larger-scale transformations.

Scheme 4. *Attempted Synthesis of the Diazo Ketone Dipeptide* **14**



a) 1.5 equiv. of HCl·H-Ala-OMe, *hv*, Et_3N (2.0 equiv.), MeCN. *b*) Aq. NaOH soln./MeOH. *c*) 1. Et_3N/$ClCO_2Et$ (1.0 equiv.), −15°; 2. CH_2N_2, −5° → r.t. *d*) 1. Et_3N/$ClCO_2Et$ (2.0 equiv.), −15°; 2. CH_2N_2, −5° → r.t. *e*) *N*-Methylmorpholine/$ClCO_2$(i-Bu) (1.0 equiv.), −15° → r.t.

products was obtained in which methyl ester **11** and the oxazolyl carbonate **13** predominated.

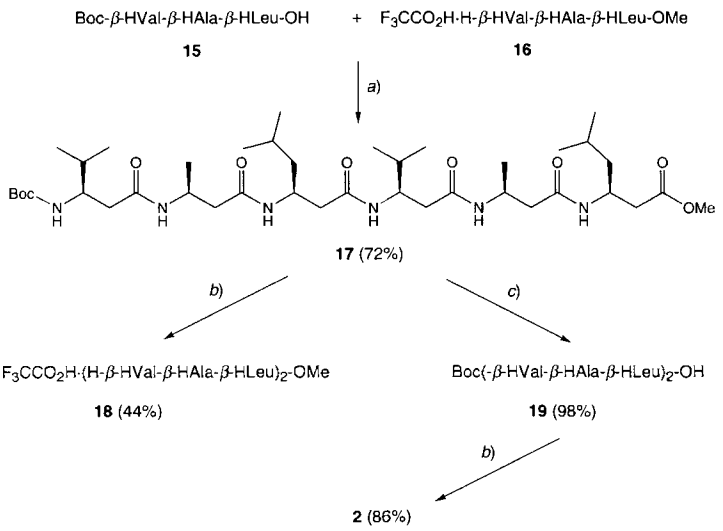
The formation of methyl ester **11** can be rationalized by assuming that the intermediate mixed anhydride has reacted with H_2O from the Et_2O solution of CH_2N_2, followed by esterification. Alternatively, reaction of the starting material (from incomplete transformation of the carboxylic acid to the mixed anhydride) with CH_2N_2 could have led to ester **11**. To preclude the last mentioned pathway, a solution of compound **12** in THF was successively treated with 2.0 equiv. of Et_3N and $ClCO_2Et$ and a solution of CH_2N_2 in Et_2O. From the obtained mixture, compound **13** was isolated in 76% yield after silica-gel chromatography[16]). Obviously, the mixed anhydride **A** has cyclized to the oxazolone **B**[17]) which reacted further with $ClCO_2Et$ to give oxazole **13**. Use of the milder base *N*-methylmorpholine in combination with $ClCO_2$(i-Bu) to activate compound **12** [10e] and subsequent addition of the mixed anhydride to a solution of CH_2N_2 in Et_2O gave a mixture of products from which the desired diazo ketone **14** was isolated in only 12% yield after silica-gel chromatography. Thus, no further attempts were made to synthesize tripeptide **1** *via* this route. In contrast to Boc-β-HVal-Ala-OH (**12**), dipeptides consisting of α-amino acids can be converted after activation of the C-terminus to their corresponding diazo ketones without complications [10d].

---

[16]) *Steglich* and *Höfle* have synthesized several 2-substituted alkyl 4-methyloxazole-5-yl carbonates [22]. However, oxazoles which contain a functionality derived from a β-amino acid at the 2-position (such as compound **13**) have previously not been described (*Chemical Abstracts* search).

[17]) Intramolecular reaction of the carbonyl O-atom of the adjacent amide with the mixed anhydride is likely to produce an oxazolone. Oxazolones are well established intermediates in peptide coupling, which can lead to racemization [23].

Scheme 5. *Fragment Coupling Towards the β-Hexapeptide* **2**



a) Et_3N, EDC, HOBt/DMF/$CHCl_3$. *b*) CF_3CO_2H. *c*) Aq. NaOH soln./CF_3CH_2OH.

The synthesis of the β-hexapeptide **2** *via* a fragment coupling is outlined in *Scheme 5*. The Boc-protected β-tripeptide ester **1** was saponified with aqueous NaOH solution to give the carboxylic acid **15** almost quantitatively, and removal of the Boc protecting group from **1** with CF_3CO_2H furnished the trifluoroacetate **16**. Subsequent activation of the carboxylic-acid function in **15** (with EDC/HOBt/Et_3N) and reaction with the free amino group of **16** produced the Boc-protected β-hexapeptide ester **17**[18])[19]). The Boc group was removed by treatment of **17** with CF_3CO_2H to yield salt **18**. Saponification of **17** with 5N NaOH in CF_3CH_2OH gave the Boc-protected β-peptide acid **19** which was converted (by treatment with CF_3CO_2H) to our synthetic target, the unprotected β-hexapeptide **2** in an overall yield of 84%. The β-hexapeptide derivatives **2** and **18** were purified by reversed-phase HPLC to produce samples which were used for conformational analysis by CD and NMR spectroscopy.

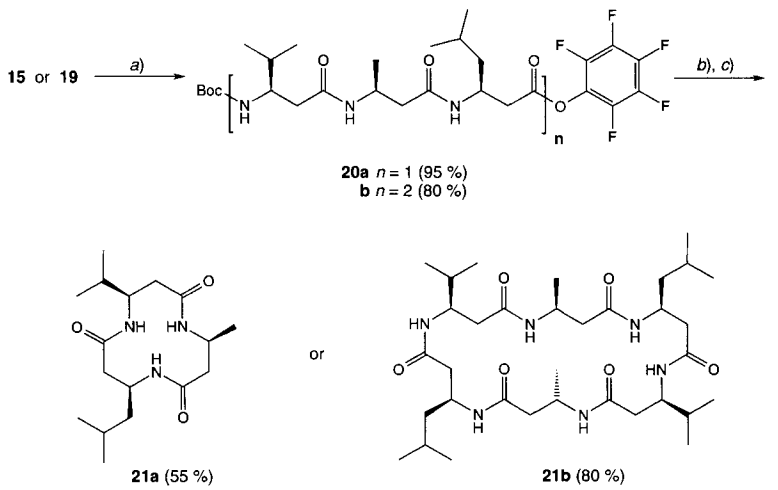
The Boc-protected β-peptides **15** and **19** were transformed into the pentafluorophenyl esters **20a** and **20b** (*Scheme 6*). Boc-Deprotection of compounds **20a,b** with CF_3CO_2H followed by slow addition of the obtained salts in MeCN to a dilute solution of 1.5 equiv. of *Hünig*'s base in the same solvent at 70° gave the cyclic compounds **21a** and **21b** in 55 and 80% yield, respectively [25].

---

[18]) Fragment coupling between peptides consisting of α-amino acids is generally troublesome because of the danger of epimerization of the activated amino-acid moiety, *via* oxazolone formation [23]. Since there is no stereogenic center in the α-carbonyl position of our β-amino acid building blocks, this problem does not exist in the assembly of β-peptides!

[19]) The Boc-β-hexapeptide ester **17** consists of enantiomerically pure β-amino acids, according to GC analysis (*Chirasil-Val*® column [11d] [24]) of volatile β-amino-carboxylic-ester derivatives obtained after hydrolysis.

Scheme 6. *Cyclization of the β-Tri- and β-Hexapeptides* **15** *and* **19** *to Cyclo-β-peptides* **21a, b**



a) Pentafluorophenol (C_6F_5OH), EDC, DMF. *b*) CF_3CO_2H. *c*) *Hünig*'s base/MeCN.

The β-tripeptide derivative **1** and the partially deprotected β-peptides **15** and **16** are well soluble in common organic solvents. Of the β-hexapeptide derivatives, the fully protected **17** is poorly soluble in MeOH[20]) and forms gels in DMSO at room temperature. Upon Boc deprotection (*i.e.* → **18**), the solubility increases dramatically (*e.g.* in $CHCl_3$), and the product **19** of ester cleavage is soluble in MeOH. The fully deprotected β-hexapeptide **2** may be dissolved in AcOH/H_2O (at pH 2), in MeOH, or in pyridine (see NMR analysis in *Sect. 3.3*). The two β-cyclopeptides **21a** and **21b** are both very insoluble[21]), except in special media such as CF_3CO_2H, CF_3CH_2OH, or 1,1,1,3,3,3-hexafluoropropan-2-ol ($(CF_3)_2CHOH$). This behavior reminds of the properties of the tube-forming α-cyclopeptides built of alternating L- and D-amino acids studied by *Lorenzi* and *Ghadiri* [25b–g] which may indicate similar membrane transporting abilities[22]).

3. Structure Analysis. – The structures of the β-peptides were investigated by CD and NMR spectroscopy, and by X-ray analysis.

3.1. *Circular Dichroism Spectroscopy.* CD Spectroscopy has been used successfully to investigate the secondary structure of peptides and proteins consisting of α-amino acids in solution; characteristic troughs and double troughs between 200 and 230 nm in the CD spectra of α-peptides and proteins are associated with β-sheet and α-helix secondary structures, respectively [26]. As mentioned in the introduction, the CD spectra of high-molecular-weight polymers of β-amino acids (only soluble in acidic or fluorinated sol-

[20]) In the isolation procedure, **17** was purified by washing with MeOH. On the other hand, the solubility in this solvent is sufficient for measuring a poor NMR spectrum.

[21]) The NMR spectrum of **21a** was recorded in $CDCl_3/CF_3CO_2H$ 3:1, the spectrum of **21b** in a 1:1 mixture of these solvents.

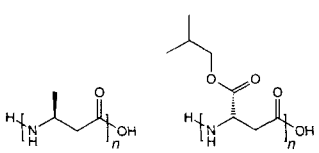
[22]) We have synthesized several (*R*)- and (*S*)-β-HAla-containing β-cyclopeptides, and we will discuss their structure and properties in detail in a separate paper.

vents) were interpreted as indicating a β-sheet-type secondary structure; the CD spectrum of poly[(*S*)-3-aminobutanoic acid] in $(CF_3)_2CHOH$ shows a large negative molar ellipticity [θ] ($-3.2 \cdot 10^3$ at 216 nm) [16d, e][23]).

An overlay of the CD spectra of dimer **10**, trimer **1**, α-hexapeptide $CF_3CO_2H \cdot$ H-Val-Ala-Leu-Val-Ala-Leu-OMe (prepared for comparison by conventional methods), and Boc-deprotected β-hexapeptide **18** in MeOH at 0.2 mM concentration is shown in *Fig. 2, a*. The α-hexapeptide $CF_3CO_2H \cdot$ H-Val-Ala-Leu-Val-Ala-Leu-OMe has a CD curve which is typical for peptides with no defined secondary structure [26]. The compounds **1** and **10** have CD curves which are similar to the spectrum of the α-hexapeptide. In contrast, the CD spectrum of the β-hexapeptide **18** shows a strong, broad minimum at 216 nm ($[\theta] = -5.2 \cdot 10^4$), a zero-crossover at 207 nm, and a maximum at 198 nm ($[\theta] = +6.2 \cdot 10^4$). As can be seen from *Figs. 2, b* and *c*, the general pattern of the CD spectrum of the β-hexapeptide does not change in different solvents (MeOH, CF_3CH_2OH, $(CF_3)_2CHOH$) or at different concentrations (0.2–0.02 mM), and it does not depend upon the protecting groups (Boc, MeO[24]); $CF_3CO_2H \cdot$ H, MeO; H, OH). On the other hand, the CD curve obtained with a solution of the β-hexapeptide **2** in aqueous H_2SO_4 solution at pH < 1 no longer shows the typical trough but is rather similar to that measured with the β-di- and β-trimer **10** and **1** in MeOH (*cf.* the corresponding curves in *Fig. 2, a* and *c*). The CD curves observed for compounds **2** and **18** strongly indicate the presence of a defined secondary structure. They show a resemblance with the CD spectra of those oligopeptides and proteins (containing α-amino acids) that are known to have a β-sheet conformation in solution [27]. The CD spectra of the β-hexapeptide derivatives are also strikingly similar to those reported for poly-β-amino acids [16d,e,j], suggesting common structural features. However, the fact that dilution does not cause major changes of the CD spectrum would indicate that we are not dealing with a secondary structure resulting from intermolecular interactions (β-sheet type) but rather one formed by intramolecular H-bonding.

A comparison of the CD spectra of the cyclic β-peptides **21a** and **21b** with those of the open-chain precursors shows a complete change upon cyclization (*Fig. 2, d*). This is especially apparent with the trimer (compare the CD spectra of **1** in *Fig. 2, a* and of **21a** in *Fig. 2, d*).

3.2. *X-Ray Crystal-Structure Analysis of Boc-β-HVal-β-HAla-β-HLeu-OMe* (**1**). Trimer **1** gave crystals from MeOH suitable for X-ray analysis. The asymmetric unit of the crystal (space group $P2_1$) contains two crystallographically independent molecules which display the same geometry, except for very small deviations in the side chains of the β-amino acids. All four C=O bonds point in the same direction. Three of the corresponding carbonyl planes are in an approximately parallel arrangement, to which the fourth



[23]) The β-peptidic polymer of isobutyl L-aspartate has a similar CD spectrum in CF_3CH_2OH but the sign is opposite ($[\theta] = +2.5 \cdot 10^4$ at 205 nm), due to the heterochiral structure as compared with L- or (*S*)-3-aminobutanoic acid.

[24]) We also measured the CD spectra of some fully protected (Boc, MeO) β-oligopeptides and of the β-di- and β-tripeptides **10** and **1** without Boc protection; only minor differences were found as compared with the spectra shown in *Fig. 2, a*.

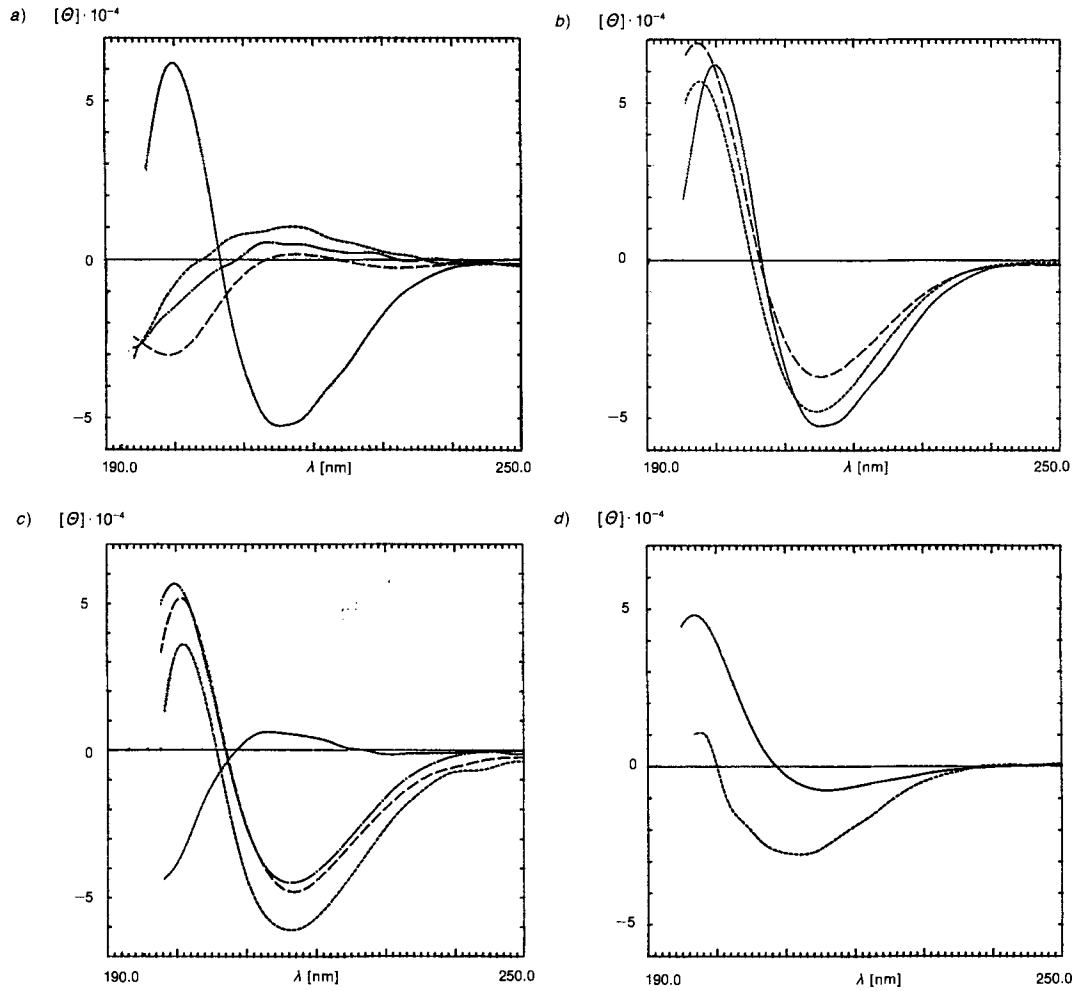


Fig. 2. *Overlay of the CD spectra* (molar ellipticity [θ] in 10 deg cm^2 mol^{-1}) *of the β-peptides* **1**, **2**, **10**, **18**, *and* **21** *under different conditions and comparison with the CD spectrum of the α-hexapeptide* $CF_3CO_2H \cdot$ *H-Val-Ala-Leu-Val-Ala-Leu-OMe. a*) Boc-β-dipeptide methyl ester **10** (–·–·–), Boc-β-tripeptide methyl ester **1** (·····), β-hexapeptide methyl ester trifluoroacetate **18** (———), and α-hexapeptide ester $CF_3CO_2H \cdot$H-Val-Ala-Leu-Val-Ala-Leu-OMe (– – – – –) in MeOH at 0.2 mM concentration. *b*) β-Hexapeptide **18** at 0.2 mM concentration in MeOH (———), CF_3CH_2OH (– – – – –), and $(CF_3)_2CHOH$ (·····). *c*) β-Hexapeptide **2** in MeOH at 0.2 mM (–·–·–), 0.04 mM (– – – – –), and 0.02 mM (·····) concentration and **2** in aq. H_2SO_4 solution at pH < 1 (———). *d*) Cyclic β-tripeptide **21a** in CF_3CH_2OH (———) and cyclic β-hexapeptide **21b** in $(CF_3)_2CHOH$ (------) at 0.2 mM concentration.

carbonyl plane is almost perpendicular, *i.e.*, the β-HVal moiety forms a turn with the rest of the molecule (*Fig. 3, a*). This turn has a resemblance with a turn observed in the so-called *Δ* fragment of crystal structures of numerous cyclic oligomers (oligolides) of β-hydroxy acids [1] [28] [29] (see superposition in *Fig. 3, b1*, right), and it can be fitted with part of the poorly resolved crystal structure [30] of the cyclic trimer of β-alanine (*Fig. 3, b2*, right). The arrangement of C=O and N–H bonds enables the trimer **1** to form a

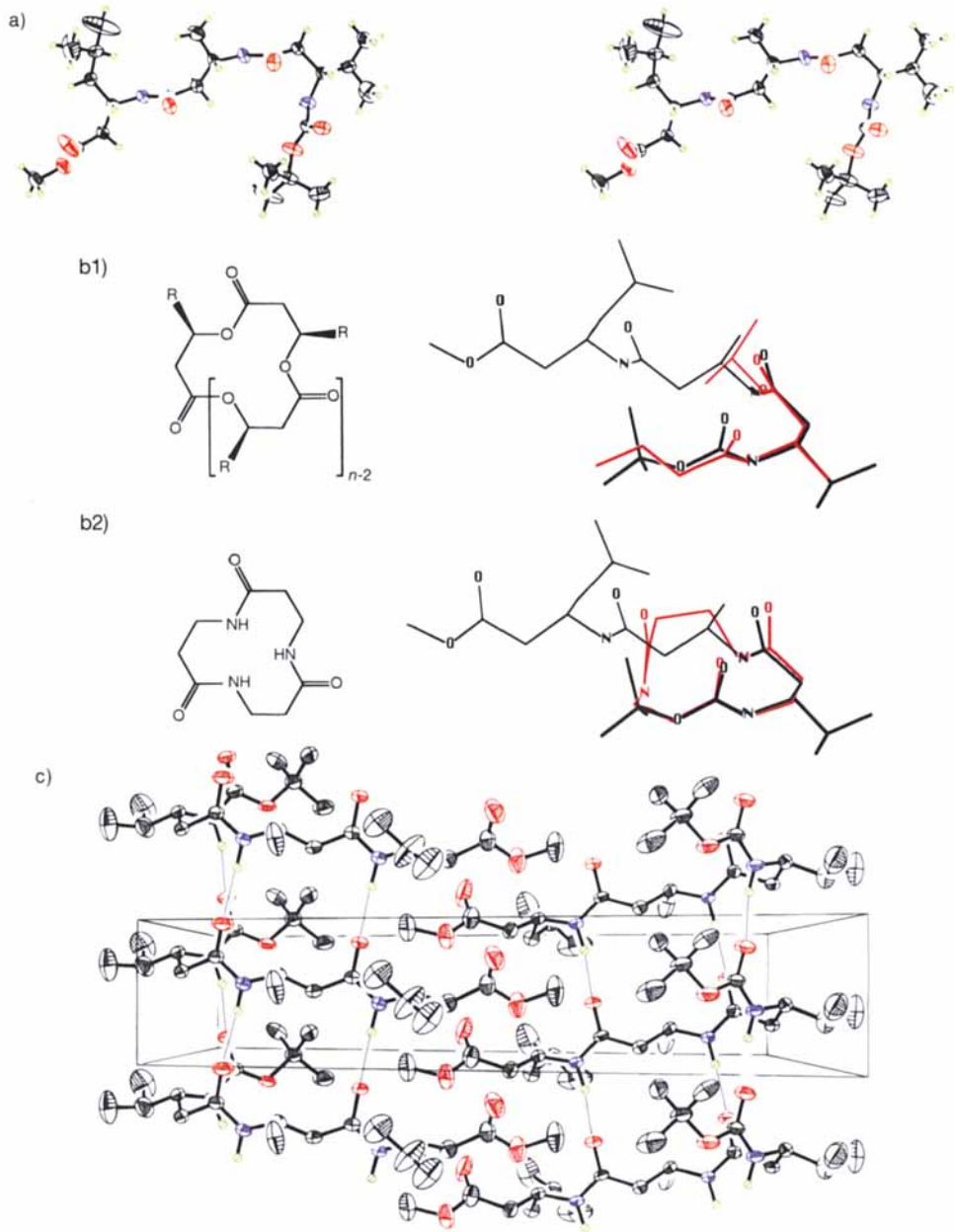


Fig. 3. *X-Ray structure of β-tripeptide* **1**: a) *Stereoscopic ORTEP representation of the structure of* **1** (thermal elipsoides are drawn to the 25% probability level, for color legend, see *Fig. 1*). b1) *Overlay of part of the Δ-fragment* (red) *as observed in the crystal structures of cyclic β-hydroxyalkanoates with the structure of* **1** (black). b2) *Formula of cyclo-tri(β-alanine) and an overlay of its poorly resolved X-ray structure* (red) [30] *with compound* **1** (black). c) *ORTEP Representation of the crystal packing of* **1** (for color legend, see *Fig. 1*; the thermal elipsoides are drawn to the 25% probability level; H-atoms bonded to C-atoms are omitted for clarity; note the parallel β-sheet type arrangement of 14-membered H-bonded rings).

close H-bonding pattern with the symmetrically equivalent molecule in the adjacent unit cell (*Fig. 3, c*). All amide and carbamate N–H and C=O groups of the β-peptide are involved. The β-HVal and β-HAla subunits form 14-membered rings (–C=O···H–N–C–C–C–N–H···O=C–N–C–C–) with the corresponding β-amino-acid moiety in the symmetrically equivalent molecule (translation along the *b*-axis) *via* H-bonds. This H-bonding pattern (N···O distance < 3 Å, see *Table 1*) is very much reminiscent of the one found in β-sheets of peptides from α-amino acids[25]).

Table 1. *H-Bond Distances and Angles of the β-Sheet-Type Structure as Observed in the X-Ray Structure of β-Trimer* **1**

	Distance H···O [Å]	Distance N···O [Å]	Angle N–H···O [°]
O(12A) N(11)	1.892	2.898	168.3
O(13A) N(12)	1.947	2.953	168.4
O(14A) N(13)	1.880	2.872	163.3
O(22A) N(21)	1.973	2.991	175.3
O(23A) N(22)	1.983	2.990	168.9
O(24A) N(23)	1.873	2.864	163.0

Thus, the crystal structure of the Boc-β-tripeptide ester **1** reveals the elements of a β-sheet-type arrangement, and, due to the bent of the β-HVal against the other two β-amino-acid residues, it might be taken as indicating a tendency of β-peptides to form turns (.....spirals, helices?!)[26]).

3.3. *NMR Structure Determination.* The structure in solution of CF_3CO_2H·H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**2**) was investigated with NMR spectroscopy using 2QF-COSY, ROESY, and TOCSY [32]. Excellent resolution of the NH signals (in the region 8.5–9.5 ppm), which simplified the signal assignments, was accomplished with (D_5)pyridine as solvent (*Table 2*). An expansion of the region of the cross-peaks as observed in the COSY spectrum between H–C(β) and the 2 H–C(α) of each β-amino-acid residue is shown in *Fig. 4, a*. In this region, one pair of cross-peaks is observed per β-amino-acid residue. In all these pairs, the coupling constants *J* between H–C(β) and each of the

Table 2. *^{1}H-NMR Chemical Shifts of the β-Hexapeptide* **2** *in (D_5)Pyridine*

β-Amino acid	HN	2 H–C(α)	H–C(β)	H–C(γ), 2 H–C(γ), or Me(γ)	Me(δ) or H–C(δ)	Me(ε)
β-HVal[1]		3.06, 2.68	3.99	2.23	1.11, 1.11	
β-HAla[2]	8.84	3.12, 2.71	5.13	1.31		
β-HLeu[3]	8.67	2.89, 2.48	4.68	1.64, 1.29	1.68	0.92, 0.85
β-HVal[4]	8.48	2.73, 2.53	4.40	1.46	0.88, 0.78	
β-HAla[5]	9.28	3.07, 2.65	4.82	1.31		
β-HLeu[6]	9.50	3.10, 2.82	4.79	1.54, 1.39	1.76	1.00, 0.89

[25]) Parallel β-sheets of normal peptides contain 12-membered rings, antiparallel β-sheets 10- and 14-membered rings, with each ring including two H-bonds [31].

[26]) Remember that both β-sheet type and helix structures have been proposed for the polymers of β-amino acids [16j, k, l].

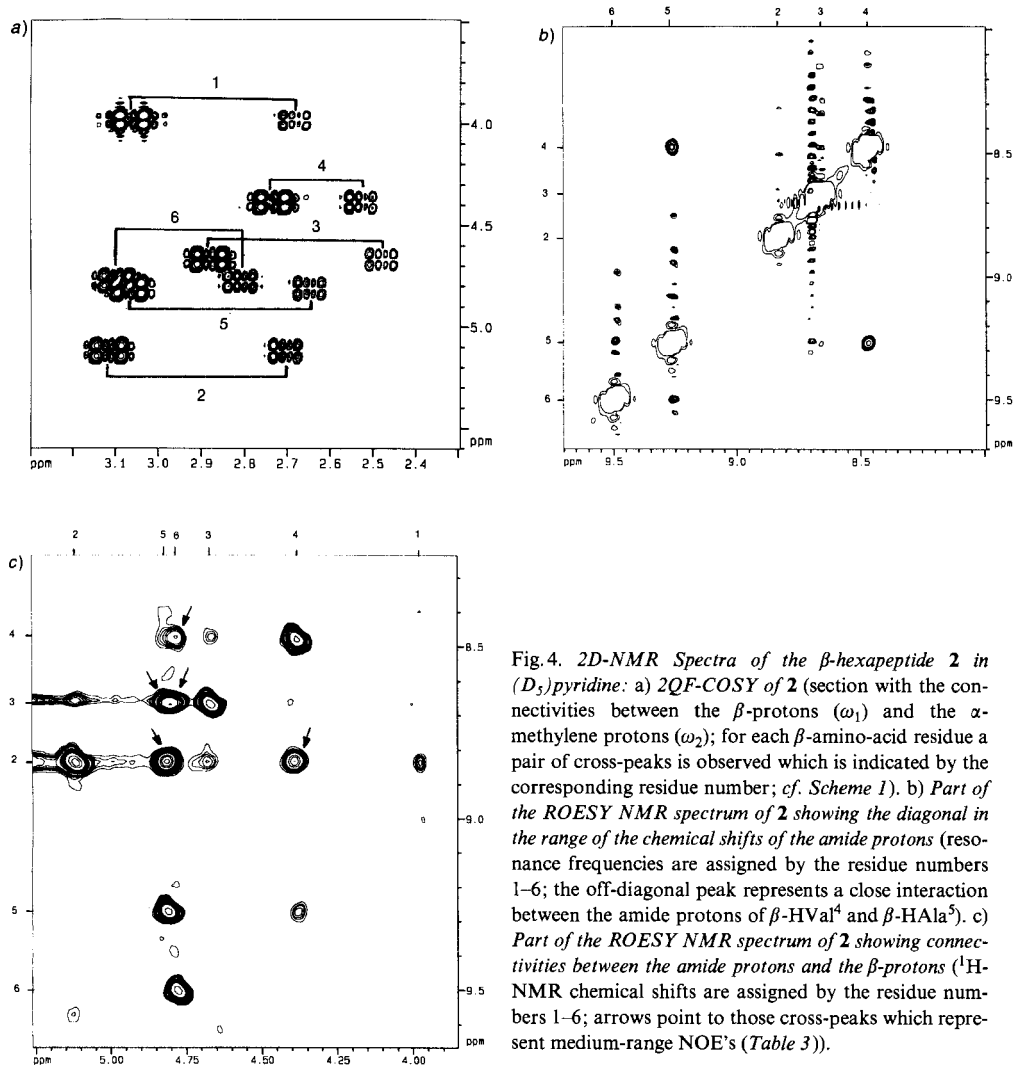


Fig. 4. *2D-NMR Spectra of the β-hexapeptide* **2** *in (D_5)pyridine:* a) *2QF-COSY of* **2** (section with the connectivities between the β-protons (ω_1) and the α-methylene protons (ω_2); for each β-amino-acid residue a pair of cross-peaks is observed which is indicated by the corresponding residue number; *cf. Scheme 1*). b) *Part of the ROESY NMR spectrum of* **2** *showing the diagonal in the range of the chemical shifts of the amide protons* (resonance frequencies are assigned by the residue numbers 1–6; the off-diagonal peak represents a close interaction between the amide protons of β-HVal[4] and β-HAla[5]). c) *Part of the ROESY NMR spectrum of* **2** *showing connectivities between the amide protons and the β-protons* (^{1}H-NMR chemical shifts are assigned by the residue numbers 1–6; arrows point to those cross-peaks which represent medium-range NOE's (*Table 3*)).

2 H–C(α) are very different. This means that there is no free rotation around the dihedral angle between HN–C(β)–C(α)–CO of the β-amino-acid residue on the NMR time scale and indicates the presence of a secondary structure.

The conformationally relevant NOE's as observed in the ROESY spectrum of the fully deprotected β-peptide **2** are listed in *Table 3*. Regions in which some of the most important NOE cross-peaks were observed are depicted in *Fig. 4, b* and *c*. In *Fig. 4, b*, an NOE of medium intensity is observed between the NH of the β-HVal[4] residue and the NH of β-HAla[5]. This is the only NH/NH NOE of the sequence. An NH/NH NOE of adjacent residues in peptides consisting of α-amino acids (*i.e.*, a d_{NN} NOE) indicates a helical structure or a turn [12c]. Several interesting connectivities are observed in the

Table 3. *Weak* (4.5 Å), *Medium* (3.5 Å), *and Strong* (3.0 Å) *NOE's as Observed in the ROESY NMR Spectrum of Compound* **2** *in (D5)Pyridine*

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

region of the ROESY shown in *Fig. 4, c*. Most notable are the NOE's between the amide proton of residue *i* and the H–C(β) of residues $i + 2$ and $i + 3$. Five NOE's of this type are possible in a hexapeptide (with an NH_2 terminus), and all of them are observed with strong or medium intensity as indicated by arrows in *Fig. 4, c*. This characteristic pattern of NOE's can be taken as a marker for the type of structure adopted by the β-hexapeptide **2** in pyridine.

For the calculation of the solution structure of β-hexapeptide **2**, a total number of 18 distance restraints (6 sequential restraints (*i.e.*, $|i-j| = 1$), 3 restraints in which $|i-j| = 2$, and 9 restraints in which $|i-j| = 3$) were derived from ROESY spectra (*Table 3*) [32]. In addition, 5 angle restraints were derived from coupling-constant measurements. A set of 20 individual structures was calculated on the basis of these restraints using standard simulated annealing protocols in X-PLOR [33]. Most of the structures converged to the same fold with no violations to the experimental distance restraints greater than 0.1 Å and dihedral restraints greater than 3 degree. The atomic root mean square deviation of 14 structures with lowest energies (< 0.5 kcal/mol) was 0.6 and 1.1 Å for backbone atoms and all heavy atoms, respectively. The structure can thus be described as well defined for the backbone but less so for the side-chain conformation. The results of these calculations are graphically depicted in *Fig. 5* with the programme WITNOTP [34], which shows stereo drawings of a top and a side view of an overlay of the 14 selected helical structures of compound **2**.

4. Enzymatic Degradation. – It has been shown that incorporation of β-amino-acid residues in peptide sequences consisting of α-amino acids can lead to increased stability against peptidases [2] [6]. Therefore, it was decided to compare the stability of the β-hexapeptide **2** and the α-hexapeptide H-Val-Ala-Leu-Val-Ala-Leu-OH against the action of pepsin [EC 3.4.23.1][27]). This enzyme was chosen because both peptide substrates are soluble in H_2O at pH 2, the optimum pH for pepsin activity. The results of our findings are depicted in *Fig. 6*. The HPLC trace of a 1:1 mixture of β-hexapeptide **2** (t_R 13.6 min) and α-hexapeptide H-Val-Ala-Leu-Val-Ala-Leu-OH (3.2 mM; t_R 10.2 min) at

[27]) Pepsin [EC 3.4.23.1] is an endopeptidase with a molecular weight of *ca.* 35000 which is secreted by the gastric mucosal cells of all vertebrates as an inactive zymogen and subsequently activated to pepsin in the stomach by limited proteolysis [35].

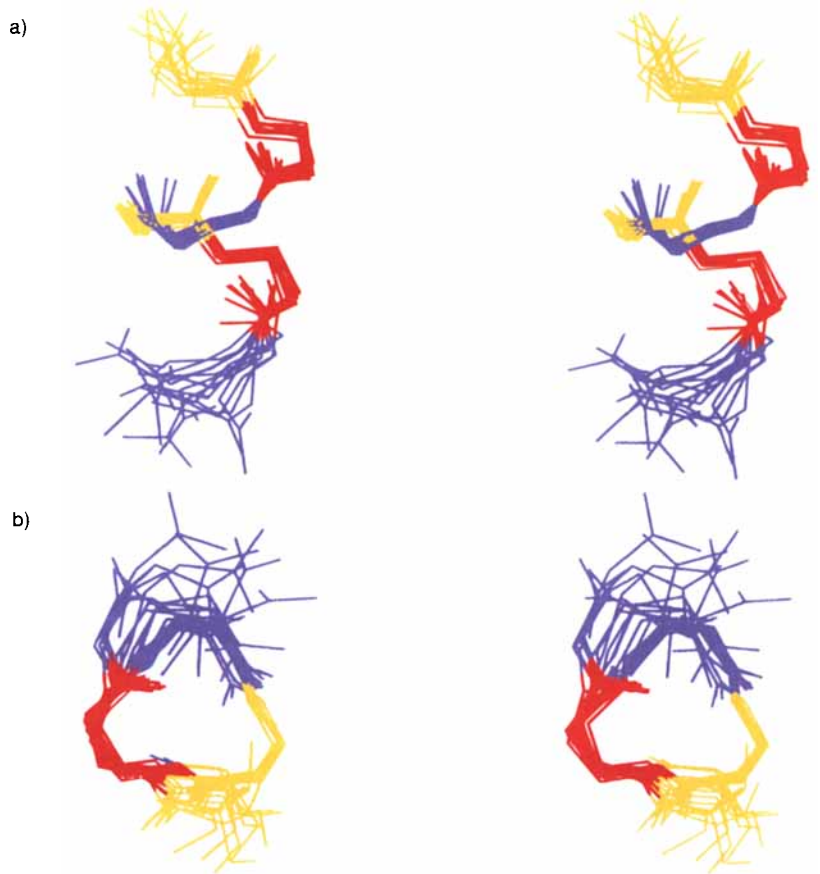


Fig. 5. *Stereo representation of an overlay of 14 selected structures of compound* **2** *with lowest energies* (< 0.5 kcal/mol). Backbone atoms (C(β), C, N) of the structural core of the molecule (β-HAla^2 to β-HAla^5) have been best-fit superpositioned with a root mean square deviations of 0.2 Å. The residues are in yellow, red, and blue for β-HVal, β-HAla, and β-HLeu, resp. For clarity, the side chain of the β-amino-acid residues are omitted. The calculated helical structure is well defined. Side and top view of the helical structures are shown in *a*) and *b*). See also *Fig. 8*.

pH 2.1 is shown in *Fig. 6*, trace *A*. Trace *B* is a chromatogram of the peptide mixture which resulted after addition of 25 u of pepsine at 37° immediately followed by removal of the enzyme *via* filtration. It can be seen that the α-hexapeptide was partially degraded into a smaller peptide with t_R 5.4 min. After addition of 250 u of pepsin to the α/β-hexapeptide mixture and incubation for 53 h at 37°, the α-peptide was completely degraded, while the β-peptide **2** was not cleaved by the enzyme, not even after this long incubation time (trace *C*)[28]). To prove that the enzyme was still active at the end of the experiment, a blank sample of pepsin was incubated for 53 h at 37° and then used to treat the α-peptide

[28]) It was shown by the use of different solvent gradients that each peak in the HPLC trace corresponds to only one compound.

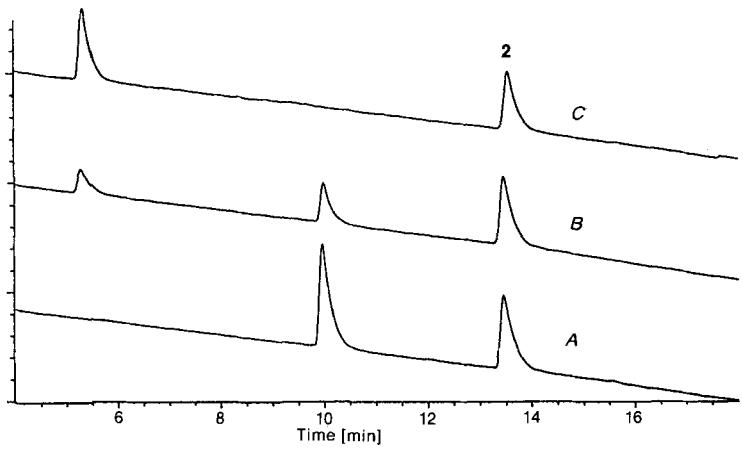


Fig. 6. *HPLC Analysis of the enzymatic degradation of the α-peptide H-(Val-Ala-Leu)$_2$-OH and of the β-hexapeptide* **2** *by pepsin. Trace A*: 1:1 mixture of the α- (t_R 10.2 min) and the β-hexapeptide (13.6 min) at pH 2.1; *Trace B*: initial 1:1 mixture of α- and β-hexapeptide after addition of 25 u pepsin [EC 3.4.23.1] at 37°, immediately followed by removal of the enzyme; *Trace C*: the 1:1 mixture of the α- and β-hexapeptide at pH 2.1 was incubated with 250 u pepsin for 53 h at 37°. For HPLC conditions, see *Exper. Part*.

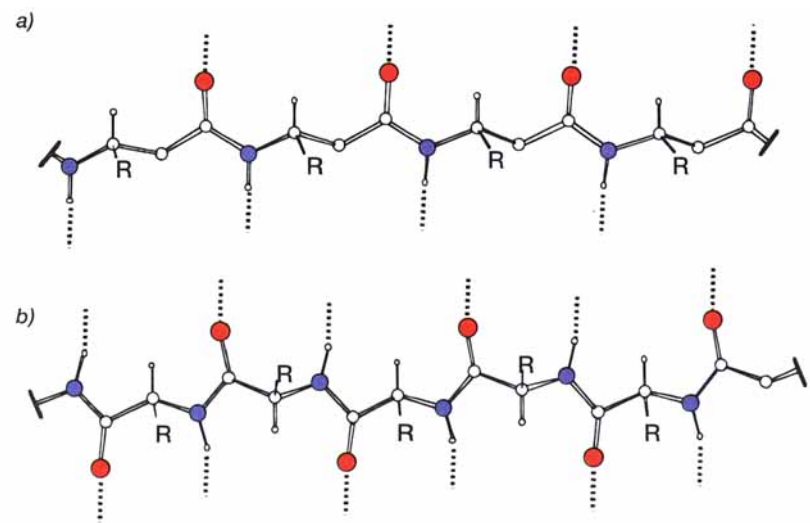


Fig. 7. *Comparison of the extended β- and α-peptide backbones*: a) *Arrangement constructed from the β-HAla-β-HLeu section in the crystal structure of* **1** (MacMoMo program by *M. Dobler*, ETH-Zürich). b) *Arrangement of a strand as found in β-sheet structures of α-peptides and proteins* (constructed with torsion angles $\phi = -119°$, $\psi = 113°$, taken from the literature [31]). The H-bonding directions are indicated by dotted lines. The β-peptide backbone has distinct faces: in one direction C=O bonds, in the opposite direction N–H bonds, approximately perpendicular C–R bonds. In contrast, in α-peptides all three types of bonds (C=O, N–H, and C–R) are alternatingly pointing in opposite directions when proceeding from one amino-acid residue to the next.

H-Val-Ala-Leu-Val-Ala-Leu-OH; the HPLC analysis proved that the enzyme completely cleaved the α-hexapeptide into the smaller peptide (data not shown). Pepsin has a preference to cleave peptides which contain the aromatic amino acids phenylalanine and tyrosine but can also cleave at other residues, such as leucine [36]. Valine and alanine residues are not particularly favorable cleavage sites [36]. Because only one cleavage product is observed upon degradation of H-Val-Ala-Leu-Val-Ala-Leu-OH, the peak at 5.4 min was tentatively assigned to result from a symmetrical cleavage producing two molecules of the tripeptide H-Val-Ala-Leu-OH. Evidence for this was provided by synthesis of a sample of this tripeptide and coinjection with the degradation product (data not shown).

5. Discussion. – *Synthesis of β-Peptides.* We have shown that the *Arndt-Eistert* sequence of reactions cannot only be used to prepare β- from α-amino acids, but also to achieve a homologation with concomitant peptide-bond formation. The Ag^+-catalyzed rearrangement has the advantage that higher concentrations may be used. The photochemical decomposition of the intermediate diazo ketones can be carried out with only small excess and with the salts of amino acid or peptide esters as nucleophiles. The necessity of handling diazomethane restricts this most attractive preparation of enantiomerically pure β-amino acids [8] to laboratory scale[29]). In our experience, the most convenient way of rapid synthesis of β-peptides is to prepare the homologated Boc-protected amino acids by decomposing the corresponding diazo ketones in aqueous THF with CF_3CO_2Ag catalysis, followed by conventional peptide coupling (EDC/HOBt). The coupling yields are generally higher than with α-amino acids, and, of course, there is no risk of epimerization involved (machine synthesis should work especially well). For the same reason, fragment coupling is an excellent way of assembling larger β-peptides from smaller ones. We have no doubt that it will be possible to construct β-peptides with functional groups in the side chains and with molecular weights approaching those of small proteins[30]).

It is also worth emphasizing that β-cyclopeptides are apparently formed with much greater ease than the normal α-cyclopeptides [25] [39][31]) (*cf.* the 55 and 80% yield of the β-cyclotri- and β-cyclohexapeptide **21a** and **21b**, resp. (*Scheme 6*), and the turn in the crystal structure of **1** in *Fig. 3*).

Finally, it is important to point out that, in our entire work with β-amino-acid derivatives, we have hitherto not encountered any problems which might be associated with β-eliminations (the functional-group pattern of β-amino acids, and thus their reactivity, is analogous to that of aldols, *cf.* also *Mannich* bases!).

Secondary Structure of β-Peptides. The biggest surprise of this investigation of β-peptides is that an oligomer containing only six amino-acid residues forms a stable helix in solution. This is in contrast to α-peptides of which distinct secondary structures are

---

[29]) The use of diazomethane requires special precautions [37]. For safety considerations, the maximum amount of diazomethane generated at a time in our laboratory is 0.2 mol.

[30]) *Cf.* the synthesis of a 128-mer from 3-hydroxybutanoic acid using fragment-coupling techniques [1] [38].

[31]) Exceptions are peptides containing proline, glycine, sarcosine, and other *N*-methyl-substituted amino acids, and those containing a certain number of D-amino acids [25] [39] (*vide infra* for the work of *Lorenzi* and *Ghadiri*).

observed in solution only when they are built of 15–20 amino-acid residues [40][32]). Thus, the naive expectation that in comparison to an α-peptide, the larger flexibility brought about by the additional CH_2 group in a β-peptide would result in a lower tendency to form secondary structures, is in fact not borne out.

From the crystal structure of the β-tripeptide derivative **1**, a β-sheet-type arrangement may be deduced[33]). As can be seen in *Fig. 7*, the appropriate backbone is fundamentally different from the one forming parallel or antiparallel β-sheets of α-peptides[34]): *i*) the C=O bonds of the β-peptide are unidirectional, and so are the N–H bonds, but in opposite directions; *ii*) all substituents R along the β-peptide backbone are located on the same side, in a 1,5-synplanar disposition; *iii*) the planes of the amide units are parallel but are diagonally translated to each other.

The turn in the crystal structure of Boc-β-HVal-β-HAla-β-HLeu-OMe (**1**; *Fig. 3, a*) might be considered as the starting point of a helix. We have pointed out the resemblance with turns present in crystal structures of cyclic oligo(3-hydroxybutanoates); using those turn fragments, we had modelled a 3_1 helix shown in *Fig. 8, a*, a structure which has not been experimentally verified with poly(3-hydroxyalkanoates)[35]). The 3_1 helix built of (*S*)-3-hydroxybutanoate units is left-handed and has a pitch of *ca.* 6 Å, and the C=O bonds are parallel to the helix axis. It turns out that the β-peptide helix of **2** identified by NMR spectroscopy has a great similarity to the hypothetical PHB helix: formal replacement of the intrachain O-atoms of the PHB helix by NH groups and the resulting C=O···H–N H-bonds lead to a 3_1 β-peptide helix with a pitch of *ca.* 5 Å. The side chains of the β-amino acids *i* and *i* + 3 reside above each other in close proximity on the outside of the helix (see *Fig. 8, b*, and compare with *Fig. 5*). The ubiquitous α-helix of normal peptides is a right-handed 3.6_1 helix (crystallographic notation) with a pitch of *ca.* 5.6 Å (*Fig. 8, c*). In the peptide nomenclature, the spiral of the β-hexapeptide is a 3_{14}, the one of the α-peptides a 3.6_{13} helix[36]).

We have, of course, no proof at this stage that the helix structure of the β-hexapeptide **2** in pyridine is also present in other solvents and that the trough in the CD spectra in MeOH of the β-hexapeptide derivatives is caused by their secondary helical structure. From the fact that the CD curve of **2** shows little concentration dependence, and from preliminary results with β-heptapeptides of the type H-β-HVal-β-HAla-β-HLeu-β-HXaa-β-HVal-β-HAla-β-HLeu-OH in which various residues β-HXaa compatible or incompatible with the 3_1-helix structure have been incorporated[37]), we may conclude that the CD minimum at *ca.* 215 nm is indeed signalling the presence of the helix.

---

[32]) Exceptions of this rule are observed *i*) with peptides containing proline or other turn-inducing amino acids [41], *ii*) with peptides containing several or only α,α-disubstituted amino acids such as the conformation-restricted 2-amino-2-methylpropanoic acid (Aib) [11] [42], and *iii*) with so-called homo-peptides consisting of sequences of the same amino acid, such as H-$(Ala)_8$-OH [26] [27b].

[33]) β-Sheet-type structures of the polymers of β-amino acids have been proposed previously by the interpretation of X-ray diffraction patterns [16b, c].

[34]) See the excellent conformational analysis of α-peptides presented in the textbook [43].

[35]) Rather, a left-handed 2_1 helix is present in the crystalline domains of poly((*R*)-3-hydroxybutyric acid) (PHB). In this helix, the C=O bonds and the C–Me bonds are approximately perpendicular to the helix axis, see the discussions in [1] [28] [29], and ref. cit. therein.

[36]) For poly(α-isobutyl-L-aspartate), a polymer of a β-amino acid with $CO_2CH_2CHMe_2$ side chains, right- and left-handed helical models with 3.25 and 4 residues per turn were proposed, based on fiber X-ray diffraction [16k, l].

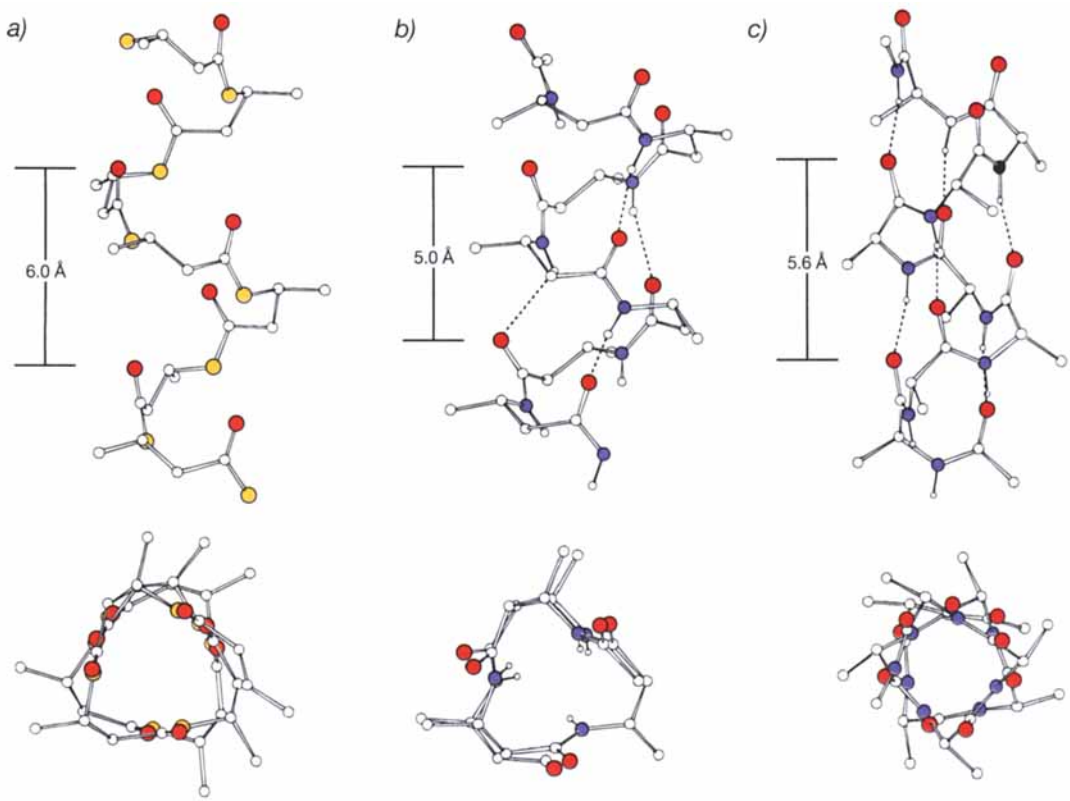


Fig. 8. *Side and top views of the helices built of (*S*)-3-hydroxy-, (*S*)-3-amino-, and (*S*)-2-aminoalkanoic acid residues* (MacMoMo presentations, the side chains are omitted for clarity). a) *Mirror image of the previously modelled* 3_1 *helix of poly(hydroxybutanoic acid)* [1] [29] (left-handed helix with a pitch of *ca.* 6 Å). b) *Left-handed* 3_1 *helix modelled from the central, well-defined section of the NMR structure of* **2** (in pyridine solution, *cf. Fig. 5*; helix with a pitch of 5 Å and almost perfect 3_1 symmetry; from the H-bonding pattern, the helix can be designated 3_{14} in the peptide nomenclature). c) *Right-handed α-helix of an α-peptide from* L*-amino acids* (3.6_{13} in the peptide nomenclature, with a pitch of 5.6 Å). For the MacMoMo modelling we used the standard torsion angles $\phi = -57°$ and $\psi = -48°$ [31]. The short pitch of the β-peptide helix and the equatorial orientation of the side chains with respect to the helical axis do not allow the incorporation of axial substituents[37]). In contrast, α,α-disubstituted α-amino acids are known to stabilize 3.6_{13}- and 3_{10}-helical structures when incorporated in α-peptides [11] [42].

6. Conclusion and Outlook. – The remarkable discovery of distinct secondary structures in β-peptides of short chain length, the ease of synthesizing β-peptides, and the observed stability to the action of a peptidase raises the following questions: Is there a world of β-peptides, β-proteins, and β-enzymes which is, in a way, orthogonal to the well known world of peptides, proteins, and enzymes built of α-amino acids? Can we increase and deepen our understanding of the chemistry and properties of α-amino-acid deriva-

[37]) Part of the projected 'tesi di laurea' of *P. Ciceri*, Università di Pavia, Italy).

tives by studying their β-amino-acid counterparts? What are the interactions[38]) between common biomolecules, cell components, living organisms and molecules with primary, secondary, and tertiary structures built entirely of β-amino acids? We have embarked in several research projects directed at gaining data which will help answering these questions.

M.O. is extremely thankful to be awarded with a 'Bundesstipendium' from the *Eidgenössische Stipendiumkommission für Ausländische Studierende*. *F.N.M.K.* thanks the *Stiftung Stipendien-Fonds der Chemischen Industrie*, Germany, for a granted scholarship. We would like to thank *A. Widmer* for help with in-house developed software tools, Dr. *C. Dalvit* for provision of NMR pulse programmes, and *Thomas Matt* for a generous donation of Val-Ala-Leu. We gratefully acknowledge the assistance of *B. Brandenberg*, *M. Bollhalder*, and Prof. *B. Jaun* (NMR service) and of Dr. *W. Amrein*, *R Häfliger*, and *O. Greter* (MS service). We thank *BASF AG*, D–Ludwigshafen, for the generous supply of THF. Continuing support by *Sandoz Pharma AG*, Basel, is gratefully appreciated.

Experimental Part

1. *Abbreviations:* EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride), FC (flash chromatography), GP (general procedure), HOBt (1-Hydroxy-1*H*-benzotriazole), h.v. (high vacuum, 0.01–0.1 Torr). Three-letter amino-acid abbrevations are used for α-amino acids Xaa; β-HXaa = β-homo-amino acid[3]).

2. *General.* THF was freshly distilled over K under Ar before use. DMF was distilled under reduced pressure from CaH_2 and stored on 4-Å molecular sieves. Et_3N and MeCN were distilled from CaH_2 and stored on KOH and 4-Å molecular sieves, resp. $ClCO_2Et$ was distilled and stored at −25°. Solvents for chromatography and for workup were distilled over *Sikkon* (anh. $CaSO_4$; *Fluka*). All other chemicals for reactions were used as purchased from *Fluka*. Amino-acid derivatives were purchased from *Bachem*, *Senn*, and *Degussa*. The diazo ketones **3**, **4**, and **6** were prepared according to the literature procedure [10d–f] [44]. The β-hexapeptide **17** was analyzed by GC, after hydrolysis and transformation to the *N*-(pentafluoropropanoyl)-substituted esters as described in [11d][19]). TLC: *Merck* silica gel *60* F_{254} anal. plates; detection with UV and I_2. FC: *Merck* silica gel *60* (40–63 μm). GC: *Chirasil-Val* column (*Macherey-Nagel*, 25 m, 0.4 mm); *Carlo-Erba-Fractovap 4160-HRGC*; injector temp. 220°; detector temp. 220° (FID); carrier gas 0.5 bar H_2; temp. program 3 min 50°, 4°/min until 180°. Anal. HPLC: *Kontron* HPLC system (UV detector *Uvikon LCD-75*, programmer *200*, integrator *Shimadzu C-R 1B Chromatopak*), *Macherey-Nagel* C_8-column (*Nucleosil 100-5* C_8 (250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump typ *64*, programmer *50*, UV-detector (variable-wavelength monitor), *Eurospher-80-*C_8-column (*Nucleosil 100-5* C_8 (250 × 20 mm). Melting points: *Büchi 510*; uncorrected. Optical rotations: 10-cm, 1-ml cell, *Perkin-Elmer-241* polarimeter (all rotations were measured at r.t.). Circular dichroism (CD): *Jobin-Yvon-Mark-III* system; recording between 190 and 300 nm; peptide concentrations 0.2, 0.04, and 0.02 mM in MeOH, CF_3CH_2OH, $(CF_3)_2CHOH$, and aq. H_2SO_4 soln. (pH < 1); molar ellipticity [θ] in deg cm^2 dmol^{-1}, λ in nm. IR spectra: *Perkin-Elmer-782* spectrophotometer. ^{1}H-NMR: *Bruker-AMX-II-500* (500 MHz), *ARX-300* (300 MHz), or *Varian-Gem-200* (200 MHz) spectrometer. ^{13}C-NMR: *Bruker-AMX-II-500* (125 MHz), *-AMX-400* (100 MHz), or *Varian-XL-300* (75 MHz) spectrometer. Mass spectra: *VG Tribrid* (EI) and *Hitachi Perkin-Elmer RMU-6M* (FAB). Elemental analyses were conducted by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

3. *General Procedure for the Synthesis of Homologated Amino-Acid Derivatives. GP Ia:* According to [10e–f], a soln. of the diazo ketone in MeOH (0.25M) at −25° (bath temp.) under Ar with the exclusion of light was treated with a soln. of silver benzoate (0.11 equiv.) in Et_3N (2.9 equiv.). The mixture was allowed to warm to r.t. within 3 h in the dark and then evaporated and the residue dissolved in AcOEt. After washing with aq. sat. $Na_2S_2O_3$, $NaHCO_3$, NH_4Cl, and NaCl soln. the org. phase was dried ($MgSO_4$) and evaporated. The residue was purified by FC.

GP Ib: A soln. of the diazo ketone in THF (0.25M) containing 10% of H_2O at −25° (bath temp.) was treated with a soln. of CF_3CO_2Ag (0.11 equiv.) in Et_3N (2.9 equiv.) under Ar with the exclusion of light. The mixture was allowed to warm to r.t. in 3 h in the dark, then diluted with Et_2O, and extracted with aq. sat. $NaHCO_3$ soln. The aq. phase was carefully adjusted to pH 2–3 at 0° with 6N HCl and extracted with Et_2O. The org. phase was dried ($MgSO_4$) and evaporated.

4. *General Procedure for the Boc-Deprotection of Amino Acids. GP IIa:* According to [45], a stirred soln. of the fully protected amino acid in CH_2Cl_2 (0.5M) was treated at 0° (ice-bath) under Ar with an equal volume of CF_3CO_2H. The mixture was allowed to warm to r.t. and stirring was continued for 1.5 h. The mixture was

[38]) There are thoroughly studied metabolisms in mammals of 3-amino-4-methylpentanoic acid (= H-β-HVal-OH), 3-aminopropanoic acid and (*R*)- and (*S*)-3-amino-2-methylpropanoic acid [6c].

evaporated and the residue dried under h.v. The salts with CF_3CO_2H were used without further purification or characterization.

GP IIb: According to [45], the Boc-protected amino acid was dissolved in CF_3CO_2H (0.25M) under Ar. After stirring for 2 h at r.t., the mixture was evaporated and the residue dried under h.v.

GP IIc: According to [45], the Boc-protected amino acid was dissolved in sat. HCl/dioxane (0.25M) at 0° (ice-bath). The mixture was allowed to warm to r.t., then stirred for 1.5 h, and evaporated. The obtained HCl salts were used without further purification.

5. *General Procedure for the Homologation of Amino Acids with Concomitant Peptide Formation. GP IIIa:* According to [10d–f], a mixture containing the free amine base amino ester (2.7 equiv.) and the Boc-protected diazo ketone (1 equiv.) in THF (0.1M) at –25° (bath temp.) was treated with a soln. of silver benzoate (0.11 equiv.) in Et_3N (2.9 equiv.) under Ar with exclusion of light. The mixture was allowed to warm to r.t. in 3 h in the dark, then diluted with Et_2O, and washed successively with aq. sat. $Na_2S_2O_3$, $NaHCO_3$, NH_4Cl, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated and the residue purified by FC.

GP IIIb: According to [10c], a stirred soln. of the amino ester trifluoroacetate (1.5 equiv.) in MeCN (0.05M) under N_2 was treated at r.t. with Et_3N (2.0 equiv.), then with a soln. of the Boc-protected diazo ketone (1 equiv.) in MeCN (0.1M). The mixture was irradiated with a low-pressure Hg lamp for 16 h, then concentrated under diminished pressure and dissolved in $CHCl_3$. After washing successively with H_2O, 1N citric acid, H_2O and aq. sat. NaCl soln., the org. phase was dried ($MgSO_4$) and evaporated. The residue was purified by FC.

6. *General Procedure for the Peptide Coupling Using EDC. GP IVa:* According to [21], a stirred soln. of the amino ester trifluoroacetate (or hydrochloride; 1 equiv.) in $CHCl_3$ (0.5M) at 0° (ice-bath) under Ar was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected amino acid (1 equiv.) in $CHCl_3$ (0.25M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t., and stirring was continued for 16 h. The mixture was diluted with $CHCl_3$ and washed with 1N HCl (thoroughly), aq. sat. $NaHCO_3$, and NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated and the residue purified using FC.

GP IVb: According to [21], a stirred soln. of the amino ester trifluoroacetate (or hydrochloride; 1 equiv.) in $CHCl_3$ (1.0M) at 0° (ice-bath) under Ar was treated with Et_3N (5 equiv.). This mixture was added to a stirred soln. of the Boc-protected amino acid (1 equiv.) in DMF (1.0M) at 0° (ice-bath) under Ar. HOBt (1.2 equiv.) and EDC (1.2 equiv.) were added successively. The mixture was allowed to warm to r.t. and stirring was continued for 16 h. The mixture was evaporated and the residue dried for 4 h under h.v. and subsequently stirred for 20 min in MeOH. The white precipitate was collected by filtration and thoroughly washed successively with MeOH and MeOH/H_2O 1:1. The product was dried for 16 h under h.v. over KOH.

7. *General Procedure for the Ester Hydrolysis. GP Va:* According to [46], a soln. of the fully protected amino acid (1 equiv.) in MeOH (1.2M) was treated with 0.75N NaOH (1.2 equiv.) at r.t. After 4 h, the mixture was adjusted to pH 2–3 with 1N HCl and extracted with AcOEt. The org. phase was dried ($MgSO_4$) and evaporated.

GP Vb: A soln. of the fully protected oligopeptide in CF_3CH_2OH (0.125M) was treated with 5N NaOH (100 equiv.) and heated at 50° (bath temp.) for 24 h. The mixture was neutralized with *Dowex-H$^+$ 50 × 8*. The ion exchanger was removed by filtration and the filtrate evaporated.

GP Vc: A soln. of the fully protected oligopeptide in CF_3CH_2OH (0.125M) was treated with 5N NaOH (5 equiv.) and stirred for 48 h at r.t. The mixture was neutralized with *Dowex-H$^+$ 50 × 8*. The ion exchanger was removed by filtration and the filtrate evaporated.

8. *General Procedure for the Synthesis of Pentafluorophenyl Esters. GP VIa:* According to [47], a soln. of the carboxylic acid derivative in DMF (0.4M) was treated at r.t. with pentafluorophenol (2.0 equiv.) and EDC (2.0 equiv.). After 16 h, the mixture was evaporated under h.v. The residue was dissolved in $CHCl_3$ and thoroughly washed with 1N HCl. The org. phase was dried ($MgSO_4$) and evaporated and the residue washed with hexane.

GP VIb: According to [47], a soln. of the carboxylic acid derivative in DMF (0.1M) was treated at r.t. with pentafluorophenol (2.0 equiv.) and EDC (2.0 equiv.). After 16 h, the mixture was concentrated under h.v. and the resulting residue dried for 4 h under h.v. and stirred for 3 h in MeOH. The white precipitate was successively washed with MeOH and MeOH/H_2O 1:1 and then dried for 16 h under h.v. over P_2O_5.

9. *General Procedure for the Cyclization of β-Peptides. GP VII:* According to [47], the β-peptide pentafluorophenyl ester trifluoroacetate was dissolved in MeCN (0.01M) and added with a syringe pump (5 ml/min) to a stirred solution of *Hünig's* base (1.5 equiv.) in MeCN (0.003M) at 70° (bath temp.). The precipitated cyclic β-peptide was collected by filtration and dried for 16 h under h.v.

10. *Solution-Structure Determination.* Assigned peaks in ROESY spectra were conservatively classified into three categories: strong, medium, and weak representing upper interproton distances of 3.0, 3.5, and 4.5 Å, resp. Dihedral restraints for 5 residues were derived from measured ^3J(NH,H–C(β)) coupling constants and incorpo-

rated during the process of structure refinement. The procedure for calculating structures followed standard simulated annealing protocols as described in the X-PLOR Manual 3.0 [33]. A starting structure for X-PLOR runs was generated with the in-house developed graphics programme WITNOTP [34]. The same programme was used to generate the topology and parameter files required for structure calculations.

11. *α-Hexapeptide CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OH. Boc-Val-Ala-Leu-Val-Ala-Leu-OMe.* Boc-Val-Ala-Leu-OMe [11d] (1.39 g, 3.36 mmol) was deprotected according to *GP IIc* and the obtained hydrochloride allowed to react with Boc-Leu-OH (0.78 g, 3.36 mmol) according to *GP IVa*. The obtained *Boc-Leu-Val-Ala-Leu-OMe* was deprotected according to *GP IIc* and the obtained hydrochloride allowed to react with Boc-Ala-OH (0.64 g, 3.36 mmol) according to *GP IVa*. The obtained *Boc-Ala-Leu-Val-Ala-Leu-OMe* was deprotected according to *GP IIc* and the obtained hydrochloride allowed to react with Boc-Val-OH (0.73 g, 3.36 mmol) according to *GP IVb*. *Boc-Val-Ala-Leu-Val-Ala-Leu-OMe* was obtained as a white amorphous solid (1.54 g, 66%). M.p. 260° (dec.). $[\alpha]_D^{r.t.} = -73.9$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3280*m*, 2960*m*, 2870*w*, 1740*w*, 1630*s*, 1530*m*, 1450*w*, 1390*w*, 1370*w*, 1250*w*, 1170*w*, 1020*w*, 670*w*. ^{1}H-NMR (200 MHz, (D_6)DMSO): 0.80–0.83 (*m*, 8 Me–C); 1.16–1.20 (*m*, 2 Me–C); 1.37–1.70 (*m*, *t*-Bu, 2 CH, 2 CH_2); 1.88–1.98 (*m*, 2 CH); 3.60 (*s*, MeO); 3.70–3.85 (*m*, 2 CH); 4.17–4.32 (*m*, 4 CH); 6.70–6.80 (*m*, NH); 7.58 (*d*, $J = 8.6$, NH); 7.90 (*d*, $J = 6.2$, NH); 8.03–8.20 (*m*, 3 NH). FAB-MS: 700.7 (37.1, $[M + 1]^+$), 699.7 (100.0, M^+), 698.0 (26.4), 599.6 (27.5), 554.5 (41.2), 498.6 (20.9), 483.5 (36.0), 428.4 (13.8), 427.4 (54.6), 384.4 (14.5), 383.4 (11.5), 328.3 (82.5), 316.3 (15.0), 284.3 (35.0), 217.2 (42.0), 213.2 (10.5), 185.2 (17.2), 171.2 (32.0), 154.1 (21.7), 116.1 (22.1).

CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OMe. Boc-Val-Ala-Leu-Val-Ala-Leu-OMe (25 mg, 0.036 mmol) was deprotected according to *GP IIb*. *CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OMe* was obtained as a white amorphous solid (23 mg, 90%). M.p. 190° (dec.). $[\alpha]_D^{r.t.} = -70.6$ ($c = 0.5$, MeOH). IR (KBr): 3290*m*, 2960*m*, 1640*s*, 1540*m*, 1460*w*, 1200*m*, 1140*m*, 835*w*, 800*w*, 720*w*, 670*w*. ^{1}H-NMR (200 MHz, CD_3OD): 0.91–1.04 (*m*, 8 Me–C); 1.27–1.38 (*m*, 2 Me–C); 1.58–1.76 (*m*, 2 CH, 2 CH_2); 2.03–2.20 (*m*, 2 CH); 3.69 (*s*, MeO); 4.20–4.27 (*m*, 2 CH); 4.38–4.46 (*m*, 4 CH); 7.94 (*d*, $J = 7.6$, NH); 8.23–8.50 (*m*, 5 NH). FAB-MS: 601.0 (70.5, $[M + 2]^+$), 600.6 (41.1, $[M + 1]^+$), 599.6 (100.0, M^+), 383.4 (12.2), 285.3 (19.1), 284.3 (40.9), 217.2 (17.8), 185.2 (16.6), 172.2 (17.9), 171.2 (53.2), 154.1 (12.0), 146.2 (22.7), 143.2 (22.5), 128.1 (12.8).

CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OH. Boc-Val-Ala-Leu-Val-Ala-Leu-OMe (300 mg, 0.43 mmol) was deprotected according to *GP Vc*, followed by *GP IIb*. *CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OH* was obtained as a white amorphous solid (210 mg, 70%). The fully protected α-peptide was purified by prep. HPLC ($MeCN/H_2O$ 35:65 (H_2O with 0.1% CF_3CO_2H)). M.p. 260° (dec.). $[\alpha]_D^{r.t.} = -51.6$ ($c = 0.5$, MeOH). IR (KBr): 3290*m*, 2965*m*, 2930*m*, 1640*s*, 1540*m*, 1460*w*, 1400*w*, 1205*m*, 1140*m*, 800*w*, 720*w*, 670*w*. ^{1}H-NMR (300 MHz, CD_3OD): 0.88–0.96 (*m*, 6 Me–C); 1.02–1.07 (*m*, 2 Me–C); 1.28–1.39 (*m*, 2 Me–C); 1.56–1.73 (*m*, 2 CH_2, 2 CH); 2.05–2.07 (*m*, CH); 2.15–2.20 (*m*, CH); 3.66–3.68 (*m*, CH); 4.21–4.23 (*m*, CH); 4.41–4.92 (*m*, 5 CH). FAB-MS: 586.6 (32.2, $[M + 1]^+$), 585.6 (100.0, M^+), 454.4 (31.6), 383.4 (14.7), 284.3 (40.8), 203.2 (11.7), 185.2 (17.6), 171.2 (54.6), 157.2 (10.9), 154.1 (14.3), 143.2 (23.1), 136.1 (12.4), 128.1 (11.4).

12. *β-Tripeptide* **1**. *Methyl (3S)-3-[(*tert*-Butoxy)carbonylamino]-5-methylhexanoate* (**5**). Diazo ketone **4** (4.0 g, 15.68 mmol) was transformed according to *GP Ia*. FC (AcOEt/hexane 15:85) yielded **5** (3.55 g, 87%). Colorless oil which solidified upon refrigeration. $[\alpha]_D^{r.t.} = -34.2$ ($c = 1.34$, $CHCl_3$; [44b]: $[\alpha]_D^{r.t.} = -22.8$ ($c = 1.47$, MeOH)). NMR: in agreement with [44a].

*(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoic Acid* (**7**). Diazo ketone **6** (2.17 g, 9.0 mmol) was transformed according to *GP Ib*; **7** (1.94 g, 93%). White microcrystalline solid. For anal. purposes, **7** was recrystallized from AcOEt. M.p. 65–66°. $[\alpha]_D^{r.t.} = -23.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3440*m*, 3010*m*, 2970*m*, 1710*s*, 1500*s*, 1390*m*, 1370*m*, 1170*s*, 1045*w*, 860*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.8$, 2 Me–C(4)); 1.42 (*s*, *t*-Bu); 1.80–1.84 (*m*, H–C(4)); 2.50–2.53 (*m*, 2 H–C(2)); 3.70–3.77 (*m*, H–C(3)); 4.95 (*d*, $J = 9.4$, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.50; 19.32; 28.35; 31.74; 37.13; 52.87; 79.49; 155.78; 177.01. EI-MS: 232.1 (< 1, $[M + 1]^+$), 188.0 (9.2), 158.0 (3.8), 140.0 (1.4), 132.0 (12.5), 116.0 (5.0), 97.0 (2.7), 88.0 (66.6) 70.0 (15.1), 57.0 (100.0), 41.0 (33.7). Anal. calc. for $C_{11}H_{21}NO_4$ (231.27): C 57.12, H 9.15, N 6.06; found: C 57.25, H 9.11, N 6.05.

Methyl (3S)-3-Amino-5-methylhexanoate (**9**). Compound **5** (0.76 g, 2.92 mmol) was Boc-deprotected according to *GP IIa*. The crude trifluoroacetate was dissolved in H_2O (0.5 ml), and Et_2O (7.0 ml) was added. The stirred mixture was adjusted to pH 8 to 0° (bath temp.) under Ar with conc. NH_3. The org. phase was washed with H_2O, dried ($MgSO_4$), and concentrated by normal-pressure distillation under Ar yielding **9** (0.36 g, 78%). Colorless oil. For anal. purposes, **9** was distilled ('Kugelrohr' apparatus). $[\alpha]_D^{r.t.} = +12.9$ ($c = 0.86$, $CHCl_3$). IR ($CHCl_3$): 2960*s*, 1730*s*, 1580*w*, 1470*m*, 1440*w*, 1370*w*, 1170*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.90 (*d*, $J = 1.6$, Me–C(5)); 0.93 (*d*, $J = 1.8$, Me–C(5)); 1.19–1.30 (*m*, H–C(5)); 1.56 (*s*, NH_2); 1.64–1.74 (*m*, 2 H–C(4)); 2.24 (*dd*, $J = 8.8$, 15.7, 2 H–C(2)); 2.46 (*dd*, $J = 4.0$, 15.7, 2 H–C(2)); 3.23–3.28 (*m*, H–C(3)); 3.70 (*s*, MeO). ^{13}C-NMR (50 MHz,

$CDCl_3$): 22.13; 23.33; 24.86; 43.06; 46.34; 47.12; 51.63; 173.39. EI-MS: 160.1 (16.9, $[M+1]^+$), 144.1 (3.9), 128.0 (2.0), 116.1 (2.2), 111.1 (8.3), 102.1 (75.5), 91.1 (13.3), 86.1 (85.2), 70.1 (49.8), 60.1 (27.9), 44.1 (100.0). Anal. calc. for $C_8H_{17}NO_2$ (159.23): C 60.35, H 10.76, N 8.80; found: C 60.51, H 10.48, N 8.67.

*Methyl (3S)-3-{(3S)-3-[(*tert*-Butoxy)carbonylamino]butanoylamino}-5-methylhexanoate* (Boc-β-HAla-β-HLeu-OMe; **10**). Following *GP IIIa:* Diazo ketone **3** (181.1 mg, 0.85 mmol) was allowed to react with **9** (360.7 mg, 2.27 mmol). FC (AcOEt/hexane 4:6) yielded **10** (246.7 mg, 84%). White microcrystalline solid.

Following *GP IIIb:* Compound **5** (281.0 mg, 1.08 mmol) was Boc-deprotected according to *GP IIa.* The obtained crude trifluoroacetate was reacted with **3** (153.4 mg, 0.72 mmol) to yield **10** after FC (174.3 mg, 70%). M.p. 122–123°. $[\alpha]_D^{r.t.} = -37.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3435*m*, 2960*m*, 1720*s*, 1700*s*, 1660*s*, 1500*s*, 1440*w*, 1370*w*, 1170*m*, 1105*w*, 1060*w*, 885*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.84–0.87 (*m*, 2 Me–C(5.1)); 1.15 (*d*, $J = 6.2$, Me–C(3.2)); 1.19–1.52 (*m*, *t*-Bu, 2 H–C(4.1), H–C(5.1)); 2.29–2.48 (*m*, 2 H–C(2.1), 2 H–C(2.2)); 3.62 (*s*, MeO); 3.86–3.94 (*m*, H–C(3.2)); 4.25–4.32 (*m*, H–C(3.1)); 5.32 (br. *s*, H–N(3.2)); 6.25 (*d*, $J = 15$, H–N(3.1)). ^{13}C-NMR (50 MHz, $CDCl_3$): 20.17; 21.72; 22.58; 24.67; 28.10; 38.79; 42.35; 42.88; 43.0; 43.93; 51.34; 78.81; 155.04; 169.97; 171.83. EI-MS: 344.2 (< 1, M^+), 288.2 (5.0), 271.2 (3.5), 243.2 (4.1), 239.2 (2.5), 229.2 (1.6), 214.1 (3.4), 201.1 (6.7), 197.1 (5.9), 188.1 (9.3), 171.1 (16.1), 160.1 (38.9), 145.1 (12.2), 130.1 (11.8), 102.1 (10.0), 88.0 (10.8), 70.0 (24.2), 59.1 (25.4), 44.1 (41.6). Anal. calc. for $C_{17}H_{32}N_2O_5$ (344.43): C 59.28, H 9.36, N 8.13; found: C 59.16, H 9.53, N 8.14.

*Methyl (3S)-3-{(3S)-3-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}butanoylamino}-5-methylhexanoate* (Boc-β-HVal-β-HAla-β-HLeu-OMe; **1**). Following *GP IIIb:* Compound **10** (1.85 g, 5.36 mmol) was Boc-deprotected according to *GP IIa.* The obtained trifluoroacetate was reacted with **6** (0.86 mg, 3.58 mmol). FC (MeOH/$CHCl_3$, 15:85) yielded **1** (1.17 g, 71%). White microcrystalline solid.

Following *GP IVa:* Boc-deprotection of **10** (3.12 g, 9.07 mmol) according to *GP IIa,* followed by reaction of the obtained trifluoroacetate with **7** (2.10 g, 9.07 mmol) gave **1** after FC (3.41 g, 82%). Crystallization from MeOH gave single crystals suitable for X-ray analysis (*Fig. 3a*). M.p. 178–179°. $[\alpha]_D^{r.t.} = -38.1$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3430*w*, 3010*m*, 2965*s*, 1710*s*, 1700*s*, 1660*s*, 1500*s*, 1440*w*, 1370*w*, 1310*w*, 1170*m*, 1020*w*, 850*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.86–0.87 (*m*, 2 Me–C(5.1), 2 Me–C(4.3)); 1.17 (*d*, $J = 6.8$, Me–C(3.2)); 1.23–1.78 (*m*, *t*-Bu, 2 H–C(4.1), H–C(4.3)); 2.27–2.50 (*m*, 2 H–C(2.1), 2 H–C(2.2), 2 H–C(2.3)); 3.64 (*s*, MeO); 3.70–3.75 (*m*, H–C(3.3)); 4.18–4.25 (*m*, H–C(3.1), H–C(3.2)); 5.19 (*d*, $J = 9.6$, H–N(3.3)); 6.58, 6.95 (2*d*, $J = 8.0$, 7.8, H–N(3.1), H–N(3.2)). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.08; 19.03; 19.40; 21.62; 22.65; 24.63; 28.10; 32.14; 39.13; 42.28; 42.78; 44.09; 44.17; 51.26; 53.12; 53.18; 78.49; 155.69; 170.19; 170.36; 171.72. FAB-MS: 458.3 (35.0, $[M+1]^+$), 380.2 (3.7), 358.3 (100.0), 281.1 (4.4), 245.2 (20.6), 193.0 (7.7), 160.2 (22.4), 154.1 (10.0), 147.1 (11.1), 136.1 (14.4), 128.1 (22.3), 116.1 (12.7), 107.0 (10.2), 91.0 (18.5), 73.0 (70.1). Anal. calc. for $C_{23}H_{43}N_3O_6$ (457.61): C 60.37, H 9.47, N 9.18; found: C 60.47, H 9.41, N 9.19.

13. *Attempted Synthesis of Diazo Ketone* **14**. *Methyl (2S)-2-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}propanoate* (Boc-β-HVal-Ala-OMe; **11**). Diazo ketone **6** (0.79 g, 3.28 mmol) was transformed according to *GP IIIb* with commercially available HCl·H-Ala-OMe (0.68 g, 4.9 mmol). FC (AcOEt/hexane 1:1) gave **11** (696.0 mg, 67%). White microcrystalline solid. M.p. 133–134°. $[\alpha]_D^{r.t.} = -20.6$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3430*w*, 3010*m*, 2970*w*, 1740*s*, 1700*s*, 1680*s*, 1500*s*, 1450*m*, 1390*w*, 1365*m*, 1310*w*, 1165*s*, 860*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.90 (*d*, $J = 2.8$, Me–C(4.2)); 0.93 (*d*, $J = 2.6$, Me–C(4.2)); 1.38 (*d*, $J = 7.2$, Me–C(2.1)); 1.42 (*s*, *t*-Bu); 1.71–1.87 (*m*, H–C(4.2)); 2.39–2.47 (*m*, 2 H–C(2.2)); 3.65–3.77 (*m*, H–C(3.2), MeO); 4.52–4.99 (*m*, H–C(2.1)); 5.03 (br. *s*, H–N(3.2)); 6.52 (br. *s*, H–N(2.1)). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.13, 18.56; 19.56; 28.55; 32.31; 39.36; 48.20; 52.56; 53.56; 79.46; 156.42; 171.24; 173.77. EI-MS: 317.20 (< 1, $[M+1]^+$), 273.2 (8.5), 260.1 (1.5), 243.1 (4.5), 217.1 (6.5), 183.1 (4.8), 173.1 (48.8), 158.1 (8.6), 145.1 (4.0), 129.1 (1.2), 116.1 (12.9); 104.1 (23.9), 98.1 (7.8), 86.1 (5.6), 72.1 (25.5), 70.0 (100.0), 57.1 (70.9), 44.1 (49.8), 41.1 (10.4). Anal. calc. for $C_{15}H_{28}N_2O_5$ (316.40): C 56.94, H 8.92, N 8.85; found: C 56.81, H 8.73, N 8.81.

*(2S)-2-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}propanoic Acid* (Boc-β-HVal-Ala-OH; **12**). Compound **11** (178.6 mg, 0.57 mmol) was transformed according *GP Va:* **12** (156.6 mg, 91%). White microcrystalline solid. M.p. 187–188°. $[\alpha]_D^{r.t.} = -22.5$ ($c = 1.0$, MeOH). IR (KBr): 3390*m*, 2975*m*, 2513*w*, 1760*m*, 1670*s*, 1625*s*, 1560*m*, 1520*s*, 1460*m*, 1420*m*, 1390*m*, 1370*m*, 1250*m*, 1220*m*, 1170*s*, 1065*w*, 780*w*. ^{1}H-NMR (200 MHz, CD_3OD): 0.89 (*d*, $J = 4.6$, Me–C(4.2)); 0.92 (*d*, $J = 4.6$, Me–C(4.2)); 1.37 (*d*, $J = 7.2$, Me–C(2.1)); 1.42 (*s*, *t*-Bu); 1.71–1.78 (*m*, H–C(4.2)); 2.28 (*dd*, $J = 8.8$, 14.2, H–C(2.2)); 2.43 (*dd*, $J = 5.2$, 14.2, H–C(2.2)); 3.73–3.77 (*m*, H–C(3.2)); 4.37 (*q*, $J = 7.2$, H–C(2.1)). ^{13}C-NMR (50 MHz, CD_3OD): 17.54; 18.06; 19.41; 28.49; 33.16; 39.46; 54.47; 79.58; 157.71; 173.24; 175.59. FAB-MS: 303.2 (33.4, $[M+1]^+$), 247.2 (20.4), 203.2 (42.0), 165.1 (11.9), 154.1 (100.0), 149.1 (13.9), 139.1 (21.8), 123.1 (22.1), 107.0 (41.1), 95.0 (47.5), 77.0 (43.5), 56.9 (91.5). Anal. calc. for $C_{14}H_{26}N_2O_5$ (302.37): C 55.61, H 8.67, N 9.26; found: C 55.48, H 8.69, N 9.21.

*2-{(2S)-2-[(*tert*-Butoxy)carbonylamino]-3-methylbutyl}-4-methyloxazol-5-yl Ethyl Carbonate* (**13**). Compound **12** (257.6 mg, 0.83 mmol) was transformed according to [10e], with 2 equiv. of Et_3N and 2 equiv. of

$ClCO_2Et$. FC (AcOEt/hexane 2:8) yielded **13** (224.1 mg, 76%). Colorless oil which solidified upon refrigeration. M.p. 48–49°. $[\alpha]_D^{r.t.} = -18.2$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3420*w*, 2980*m*, 1780*s*, 1705*s*, 1580*w*, 1500*s*, 1390*w*, 1370*m*, 1170*m*, 1040*w*, 990*w*, 960*m*, 900*w*, 890*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.92 (*d*, $J = 6.8$, Me–C); 0.94 (*d*, $J = 6.8$, Me–C); 1.40 (*t*, $J = 7.15$, CH_2); 1.41 (*s*, *t*-Bu); 1.71–1.78 (*m*, CH); 2.03 (*s*, Me); 2.77–2.96 (*m*, 2 CH); 3.79–3.82 (*m*, CH); 4.35 (*q*, $J = 7.15$, Me); 4.83 (*d*, $J = 9.35$, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 10.0 (Me); 14.01 (CH_2); 18.17 (Me); 19.29 (Me); 28.30 (Me); 31.47 (CH_2); 31.52 (CH); 53.98 (CH); 66.19 (Me); 79.11 (C); 118.48 (C); 145.88 (C); 151.47 (C); 155.40 (C); 155.46 (C). EI-MS: 357.2 (< 1, $[M + 1]^+$), 283.1 (3.4), 228.1 (7.7), 210.1 (10.7), 195.1 (4.2), 185.1 (6.0), 167.1 (15.2), 144.1 (20.4), 141.1 (14.1), 116.1 (51.9), 113.0 (61.5), 98.1 (17.0), 72.1 (100.0), 57.1 (40.5), 42.1 (4.7). Anal. calc. for $C_{17}H_{28}N_2O_6$ (356.41); C 57.29, H 7.92, N 7.86; found: C 56.09, H 7.79, N 7.74.

14. *β-Hexapeptide* **2**. *(3S)-3-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}-butanoylamino}-5-methylhexanoic Acid* (Boc-β-HVal-β-HAla-β-HLeu-OH; **15**). Compound **1** (390 mg, 0.85 mmol) was transformed according to *GP Va*: **15** (364.2 mg, 97%). White microcrystalline solid. M.p. 190–191°. $[\alpha]_D^{r.t.} = -8.87$ ($c = 0.53$, MeOH). IR (KBr): 3315*m*, 2960*m*, 1685*s*, 1655*s*, 1650*s*, 1640*s*, 1560*s*, 1540*s*, 1490*m*, 1460*m*, 1365*m*, 1175*m*. ^{1}H-NMR (200 MHz, CD_3OD): 0.87–0.92 (*m*, 2 Me–C(5.1), 2 Me–C(4.3)); 1.15 (*d*, $J = 6.8$, Me–C(3.2)); 1.28–1.74 (*m*, *t*-Bu, 2 H–C(4.1), H–C(5.1), H–C(4.3)); 2.12–2.49 (*m*, 2 H–C(2.1), 2 H–C(2.2), 2 H–C(2.3)); 3.71–3.76 (*m*, H–C(3.3)); 4.12–4.30 (*m*, H–C(3.1), H–C(3.2)). ^{13}C-NMR (50 MHz, CD_3OD): 18.42; 19.75; 20.06; 22.23; 23.71; 26.12; 28.90; 33.84; 40.34; 41.06; 43.92; 44.60; 44.70; 45.98; 54.95; 79.96; 158.05; 172.53; 173.19; 174.86. FAB-MS: 444.29 (56.2, $[M + 1]^+$), 366.2 (12.3), 344.2 (75.0), 231.2 (16.8), 154.1 (23.8), 136.0 (17.5), 128.1 (19.9), 95.0 (10.3), 86.0 (15.1), 72.0 (26.7), 56.9 (45.1). Anal. calc. for $C_{22}H_{41}N_3O_6$ (443.58): C 59.57, H 9.32, N 9.47; found: C 59.28, H 9.44, N 9.44.

Methyl N-*[(*tert*-Butoxy)carbonyl]-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucyl-(*R*)-β-homovalyl-(*S*)-β-homoalanyl-(*S*)-β-homoleucinate* (Boc(-β-HVal-β-HAla-β-HLeu)$_2$-OMe; **17**). Compound **1** (390 mg, 0.85 mmol) was Boc-deprotected according to *GP IIa*. The obtained trifluoroacetate **16** was coupled with **15** (377.0 mg, 0.85 mmol) as described in *GP IVb*: **17** (479.6 mg, 72%). White amorphous solid. For anal. purposes, **17** was precipitated from CF_3CH_2OH/EtOH. M.p. 240° (dec.). $[\alpha]_D^{r.t.} = -34.6$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3300*m*, 3080*w*, 2960*m*, 1690*s*, 1650*s*, 1540*s*, 1460*w*, 1370*m*, 1310*w*, 1250*w*, 1175*m*, 1150*w*, 1050*w*, 1030*w*. ^{1}H-NMR (200 MHz, CD_3OD): 0.81–0.92 (*m*, 2 Me–C(5.1), 2 Me–C(5.4), 2 Me–C(4.3), 2 Me–C(4.6)); 1.12–1.15 (*m*, Me–C(3.2), Me–C(3.5)); 1.33–1.80 (*m*, *t*-Bu, 2 H–C(4.1), H–C(5.1), H–C(4.3), 2 H–C(4.4), H–C(5.4), H–C(4.6)); 2.20–2.46 (*m*, 2 H–C(2.1–6)); 3.65 (*s*, MeO); 3.70–4.40 (*m*, H–C(3.1–6)). ^{13}C-NMR (50 MHz, 100°, (D_6)DMSO): 17.0; 18.06; 18.5; 19.13; 19.83; 19.88; 21.81; 21.94; 23.02; 23.18; 23.9; 24.52; 25.0; 28.41; 29.5; 31.28; 31.92; 41.82; 42.42; 42.5; 42.55; 42.67; 43.39; 43.44; 44.39; 44.84; 51.4; 53.36; 77.65; 155.52; 169.57; 169.78; 169.91; 170.02; 171.47; 172.0. MALDI-MS: 805.0 ($[M + Na]^+$). Anal. calc. for $C_{40}H_{74}N_6O_9$ (783.06): C 61.35, H 9.53, N 10.73; found: C 61.33, H 9.68, N 10.64.

Methyl (R)-β-Homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate Trifluoroacetate (H(-β-HVal-β-HAla-β-HLeu)$_2$-OMe·CF_3CO_2H; **18**). Compound **17** (36 mg, 0.046 mmol) was Boc-deprotected according to *GP IIb* and purified by prep. reversed-phase HPLC (MeCN/H_2O 35:65) (H_2O containing 0.1% CF_3CO_2H): **18** (15.1 mg, 44%). Colorless foam. The peptide was precipitated as an amorphous solid from MeCN. M.p. 220–222°. $[\alpha]_D^{r.t.} = +6.6$ ($c = 1.0$, $CHCl_3$). CD (0.2 mM in MeOH): $+6.20 \cdot 10^4$ (198), $-5.24 \cdot 10^4$ (216.0). IR ($CHCl_3$): 3350*w*, 3270*m*, 2960*m*, 1725*m*, 1650*s*, 1555*m*, 1440*w*, 1375*w*, 1290*w*, 1260*w*, 1175*m*, 1135*w*, 1100*w*. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$ 8:2): 0.73–0.86 (*m*, 2 Me–C(5.1), 2 Me–C(4.3), 2 Me–C(5.4)); 0.95–0.98 (*m*, 2 Me–C(4.6)); 1.03, 1.08 (2*d*, $J = 6.3$, 6.6, Me–C(3.2), Me–C(3.5)); 1.11–1.56 (*m*, 2 H–C(4.1), H–C(5.1), H–C(4.3), 2 H–C(4.4), H–C(5.4)); 1.91–1.98 (*m*, H–C(4.6)); 2.10–2.7 (*m*, 2 H–C(2.1–6)); 3.37–3.41 (*m*, CH); 3.56 (*s*, MeO); 3.95–4.03 (*m*, CH); 4.21 (*m*, 3 CH); 4.48–4.52 (*m*, CH); 7.44 (*d*, $J = 9.6$, NH); 7.90–8.13 (*m*, 2 NH); 8.15 (*d*, $J = 9.6$, NH). ^{13}C-NMR (75 MHz, CD_3OD): 18.15; 19.17; 19.37; 19.41; 20.77; 20.91; 21.04; 22.84; 22.89; 23.36; 23.65; 23.82; 25.86; 26.04; 26.04; 32.02; 34.07; 36.12; 39.01; 40.49; 40.68; 42.01; 43.01; 43.18; 43.34; 43.59; 45.16; 45.80; 46.20; 46.31; 118.0 (*q*); 161.6 (*q*); 171.57; 171.87; 172.72; 173.16; 174.15; 186.51. FAB-MS: 684.8 (25.9, $[M + 1]^+$), 683.5 (100.0, M^+), 245.2 (8.3), 182.2 (11.4), 154.1 (11.6), 128.1 (26.2), 111.1 (11.9), 97.1 (12.9), 86.1 (25.6), 72.1 (41.8), 69.0 (24.9), 55.0 (16.0).

N-*[(*tert*-Butoxy)carbonyl)-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucine* (Boc(-β-HVal-β-HAla-β-HLeu)$_2$-OH; **19**). Compound **17** (65.0 mg, 0.083 mmol) was transformed as described in *GP Vb*: **19** (62.3 mg, 98%). White amorphous solid. For anal. purposes, **19** was precipitated from CF_3CH_2CH/MeOH. M.p. 240° (dec.). $[\alpha]_D^{r.t.} = -14.8$ ($c = 0.5$, MeOH). IR (KBr): 3300*m*, 3105*w*, 2960*m*, 1720*w*, 1685*s*, 1655*s*, 1560*m*, 1540*m*, 1510*w*, 1500*w*, 1460*w*, 1365*m*, 1175*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.88–0.93 (*m*, 2 Me–C(5.1), 2 Me–C(4.3), 2 Me–C(5.4), 2 Me–C(4.6)); 1.14 (*d*, $J = 6.8$, Me–C); 1.15 (*d*, $J = 6.6$, Me–C); 1.30–1.80 (*m*, *t*-Bu, 2 H–C(4.1), H–C(5.1), H–C(4.3), 2 H–C(4.4), H–C(5.4), H–C(4.6));

2.22–2.45 (*m*, 2 H–C(2.1–6)); 3.75 (*m*, H–C(3.6)); 4.00–4.41 (*m*, H–C(3.1–5)). ^{13}C-NMR (50 MHz, CD_3OD): 18.36; 19.27; 21.95; 23.34; 23.41; 25.74; 25.79; 28.46; 28.52; 32.78; 40.71; 42.64; 43.0; 43.5; 44.06; 44.17; 44.37; 44.46; 45.54; 47.06; 51.0; 52.0; 54.0; 61.0; 75.0; 83.0; 163.0; 172.06; 172.11; 172.26; 172.3; 172.34; 177.66. FAB-MS: 791.6 (93.3, $[M + Na]^+$), 769.4 (14.6, $[M + H]^+$), 691.5 (11.4), 669.4 (100.0), 556.4 (19.8), 471.3 (9.7), 344.2 (17.7), 255.2 (8.8). Anal. calc. for $C_{39}H_{72}N_6O_9$ (769.0): C 60.91, H 9.44, N 10.93; found: C 60.90, H 9.53, N 10.75.

(R)-β-Homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucine Trifluoroacetate (H(-β-HVal-β-HAla-β-HLeu)$_2$-OH·CF_3CO_2H; **2**). Compound **19** (76.0 mg, 0.099 mmol) was Boc-deprotected according to *GP IIb*. The oily residue was triturated with Et_2O: **2** (66.5 mg, 86%). Colorless foam. Peptide **2** was purified by prep. reversed-phase HPLC (MeCN/H_2O 35:65, (H_2O containing 0.1% CF_3CO_2H)). $[\alpha]_D^{r.t.} = +6.3$ ($c = 1.0$, MeOH). CD (0.2 mM in MeOH): $+5.90 \cdot 10^4$ (200), $-4.50 \cdot 10^4$ (216.0). IR (KBr): 3290*m*, 3100*w*, 2965*m*, 1655*s*, 1640*s*, 1560*m*, 1460*w*, 1200*m*, 1190*m*, 1140*m*, 720*m*. ^{1}H-NMR (300 MHz, CD_3OD): 0.86–0.93 (*m*, 2 Me–C(5.1), 2 Me–C(4.3), 2 Me–C(5.4)); 1.07 (*d*, $J = 6.6$, 2 Me–C(4.6)); 1.14, 1.16 (2*d*, $J = 15.6$, Me–C(3.2), Me–C(3.5)); 1.24–1.69 (*m*, 2 H–C(4.1), H–C(5.1), H–C(4.3), 2 H–C(4.4), H–C(5.4)); 1.95–2.05 (*m*, H–C(4.6)); 2.19–2.74 (*m*, 2 H–C(2.1–6)); 3.48–3.51 (*m*, CH); 4.14–4.16 (*m*, H–C); 4.33–4.52 (*m*, 4 CH). ^{13}C-NMR (300 MHz, CD_3OD): 17.81; 18.83; 19.05; 19.24; 20.51; 20.78; 22.53; 23.06; 23.36; 25.68; 31.68; 33.55; 35.76; 38.83; 40.5; 41.54; 42.63; 42.97; 43.16; 43.32; 45.08; 45.29; 45.40; 45.99; 52.68; 55.97; 120.0 (*q*); 162.0 (*q*); 171.2; 171.26; 171.7; 172.19; 172.76; 174.6. FAB-MS: 670.6 (19.6, $[M + 1]^+$), 669.4 (73.2, M^+), 365.2 (11.0), 231.2 (8.8), 212.2 (5.1), 200.2 (5.4).

15. *Cyclic β-Peptides* **21a,b**. *Pentafluorophenyl(3S)-3-{(3S)-3-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}butanoylamino}-5-methylhexanoate* (Boc-β-HVal-β-HAla-β-HLeu-OC_6F_5; **20a**). Compound **15** (107.8 mg, 0.24 mmol) was transformed according to *GP VIa*; **20a** (139.5 mg, 95%). White amorphous solid. M.p. 140–142°. $[\alpha]_D^{r.t.} = -26.7$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 3030*m*, 2965*m*, 1785*w*, 1665*m*, 1519*s*, 1430*w*, 1370*w*, 1310*w*, 1100*w*, 1000*m*, 930*m*, 850*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.88–0.95 (*m*, 2Me–C(5.1), 2 Me–C(4.3)); 1.21 (*d*, $J = 6.8$, Me–C(3.2)); 1.34–1.78 (*m*, *t*-Bu, 2 H–C(4.1), H–C(4.3), H–C(5.1)); 2.30–2.42 (*m*, 2 H–C(2.2), 2 H–C(2.3)); 2.85–2.93 (*m*, 2 H–C(2.1)); 3.68–3.70 (*m*, CH); 4.40–4.44 (*m*, CH); 4.95 (*d*, $J = 9.6$, NH(3.3)); 6.62 (*d*, $J = 8.4$, NH); 6.94 (*d*, $J = 6.2$, NH). FAB-MS: 1219.3 (2.3, $[2\ M + 1]^+$), 643.2 (19.0), 632.1 (11.1), 610.1 (34.4, $[M + 1]^+$), 511.1 (27.6), 510.1 (100.0), 397.0 (14.6), 243.1 (3.7), 170.1 (3.0), 154.0 (5.5), 128.0 (11.0).

Pentafluorophenyl N-*[(*tert*-Butoxy)carbonyl]-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucyl-(R)-β-homovalyl-(S)-β-homoalanyl-(S)-β-homoleucinate* (Boc-(β-HVal-β-HAla-β-HLeu)$_2$-OC_6F_5; **20b**). Compound **19** (357.8 mg, 0.46 mmol) was transformed according to *GP VIb*; **20b** (342.1 mg, 80%). White amorphous solid. M.p. 250° (dec.). $[\alpha]_D^{r.t.} = -37.8$ ($c = 0.5$, CF_3CH_2OH). IR (KBr): 3310*m*, 3080*w*, 2960*m*, 1790*w*, 1690*m*, 1650*s*, 1520*s*, 1460*w*, 1370*m*, 1310*w*, 1250*w*, 1175*w*, 1090*w*, 1000*m*. ^{1}H-NMR (200 MHz, CD_3OD): 0.85–0.97 (*m*, 2 Me–C(5.1), 2 Me–C(5.4), 2 Me–C(4.3), 2 Me–C(4.6)); 1.13–1.17 (*m*, Me–C(3.2), Me–C(3.5)); 1.20–1.75 (*m*, *t*-Bu, 2 H–C(4.1), H–C(5.1), H–C(4.3), 2 H–C(4.4), H–C(5.4), H–C(4.6)); 2.21–2.43 (*m*, 2 H–C(2.2–6)); 2.89–2.92 (*m*, 2 H–C(2.1)); 3.65–3.80 (*m*, H–C); 4.00–4.50 (*m*, 5 CH). FAB-MS: 936.4 (14.0, $[M + 1]^+$), 935.4 (27.8, M^+), 924.5 (12.8), 837.3 (14.3), 836.3 (51.2), 835.3 (100.0), 722.2 (21.7), 669.3 (10.3), 637.2 (11.1), 510.1 (10.8), 397.1 (14.0), 243.1 (11.5), 225.1 (11.6), 224.1 (13.2), 196.1 (16.9), 182.1 (25.4), 170.1 (17.7), 154.1 (33.8), 136.0 (26.4), 128.0 (44.6), 111.0 (30.3).

Cyclo(-β-HVal-β-HAla-β-HLeu-) (**21a**). Compound **20a** (139.5 mg, 0.23 mmol) was Boc-deprotected according to *GP IIa*. The obtained trifluoroacetate was transformed according to *GP VII*: **21a** (41.4 mg, 55%). White amorphous solid. M.p. 300° (dec.). $[\alpha]_D^{r.t.} = -8.8$ ($c = 0.5$, CF_3CH_2OH). IR (KBr): 3295*s*, 3080*w*, 2960*m*, 2875*w*, 1650*s*, 1560*s*, 1450*m*, 1370*m*, 1305*w*, 1280*w*, 1205*m*, 1145*w*, 1110*w*, 1075*w*, 1050*w*, 980*w*, 690*m*. ^{1}H-NMR (500 MHz, CF_3CO_2H/CD_3Cl_3 8:2): 0.89–0.95 (*m*, 4 Me–C); 1.32 (*d*, $J = 6.7$, Me–C); 1.35–1.40 (*m*, CH); 1.50–1.58 (*m*, CH_2); 1.78–1.82 (*m*, CH); 2.46–2.63, 2.79–2.88 (*m*, 3 CH_2); 4.28 (br. *m*, CH); 4.59 (br. *m*, 2 CH); 7.74 (br. *m*, NH); 7.89 (br. *m*, NH); 8.24 (br. *m*, NH). ^{13}C-NMR (125 MHz, $CF_3CO_2H/CDCl_3$ 8:2): 17.77; 18.16; 20.02; 21.27; 22.06; 24.84; 32.56; 38.18; 41.28; 42.05; 43.63; 46.09; 48.42; 54.78; 174.26; 174.47; 175.19. FAB-MS: 976.7 (1.7, $[3M + 1]^+$), 651.4 (23.6, $[2\ M + 11]^+$), 349.2 (14.0), 348.2 (47.0), 327.2 (34.0), 326.2 (100.0, $[M + 1]^+$), 324.2 (18.3), 308.2 (9.0), 200.1 (12.6), 182.1 (14.2), 154.1 (11.0), 128.1 (15.4), 112.0 (12.6), 111.0 (14.20).

Cyclo(-β-HVal-β-HAla-β-HLeu-β-HVal-β-HAla-β-HLeu-) (**21b**). Compound **20b** (342.1 mg, 0.37 mmol) was Boc-deprotected according to *GP IIb*. The obtained trifluoroacetate was transformed according to *GP VII*: **21b** (192.4 mg, 80%). White amorphous solid. M.p. 300° (dec.). $[\alpha]_D^{r.t.} = +19.0$ ($c = 0.5$, CF_3CO_2H). IR (KBr): 3300*s*, 3085*w*, 2960*m*, 2870*w*, 1700*s*, 1650*s*, 1540*s*, 1450w, 1370*w*, 1305*w*, 1260*w*, 1200*w*, 1140*w*, 985*w*, 700*w*. ^{1}H-NMR (500 MHz, $CF_3CO_2H/CDCl_3$ 1:1): 0.89–0.98 (*m*, 8 Me–C); 1.22–1.30 (*m*, 2 Me–C); 1.39–1.45 (*m*, 2 CH); 1.54–1.61 (*m*, 2 CH_2); 1.84–1.93 (*m*, 2 CH); 2.57–2.85 (*m*, 6 CH_2); 4.01–4.10 (*m*, 2 CH); 4.34–4.40 (*m*, 4 CH); 7.86

(*d*, J = 8.2, NH); 8.00 (*d*, J = 8.3, NH); 8.08 (*d*, J = 7.1, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.55; 17.97; 19.18; 21.06; 21.92; 25.03; 32.16; 37.79; 40.67; 41.35, 43.29; 45.75; 47.94; 55.22; 174.75; 175.30; 175.44. FAB-MS: 1476.6 (16.5), 1475.4 (48.7), 1320.1 (30.9), 803.5 (22.1), 750.7 (10.4), 749.5 (43.8), 689.4 (18.0), 673.5 (16.1), 670.7 (29.2), 669.5 (100.0), 651.5 (30.6, $[M + 1]^+$), 613.2 (20.7), 524.3 (3.1).

16. *Enzymatic Degradation.* A mixture of CF_3CO_2H·H-Val-Ala-Leu-Val-Ala-Leu-OH (3.0 mg, 0.005 mmol) and **2** (3.0 mg, 0.004 mmol) was dissolved in conc. AcOH (0.3 ml) at r.t. and diluted with 1.74N AcOH (7.5 ml) to give a soln. of pH 2.1. This soln. was divided into 3 parts of 2.6 ml, each being shaken in a test tube at 37° for 10 min and then treated with 25 and 250 u, resp., of pepsine [EC 3.4.23.1] dissolved in 1.74M AcOH (0.5 ml). After the incubation time, the enzyme was removed by centrifugation (1 h, 5000 g) over a *Centricon 10* filter. The filtrate was analyzed by reversed-phase HPLC (*Nucleosil 100-5 C_8* (250 × 4 mm), flow 1.0 ml/min; 60°; gradient of MeCN/H_2O (H_2O containing 0.1% CF_3CO_2H): 0.01 min 1:9, 16.00 min 1:1, 18.00 min 1:1, and 21.00 min 1:9.

17. *Crystal-Structure Analyses. (3S)-3-[(*tert*-Butoxy)carbonylamino]-1-diazo-5-methylhexan-2-one* (**4**; $C_{12}H_{20}N_3O_3$) [44a]. Determination of the cell parameters and collection of the reflection intensities were performed on an *Enraf-Nonius-CAD4* four-circle diffractometer (graphite monochromatized MoK_α radiation, λ = 0.7107 Å). Colorless prism, 0.3 × 0.4 × 0.9 mm, triclinic, space group $P1$, a = 9.116 (5) Å, b = 9.197 (6) Å, c = 10.864 (6) Å, α = 70.29 (4)°, β = 77.58 (4)°, γ = 63.47 (3)°, V = 769.1 (6) Å^3, Z = 2, $\rho_{calc.}$ = 1.098 gcm^{-3}, μ = 0.08 mm^{-1}, $F(000)$ = 274. Number of reflections measured 2849 ($\omega/2\theta$ scan, $2 < 2\theta < 50°$, T 295 K); 2690 unique reflections, of which 2047 with $I > 3\sigma(I)$ were used for the determination (direct methods, SHELXS-86). SHELXL-93 was used for structure refinement (full-matrix least-squares). The non-H-atoms were refined anisotropically, the H-atoms were added to the molecule with constant isotropic temp. factors on idealized positions and refined according to the riding model (afix 3). The refinement converged at R = 0.053 (wR^2 = 0.145), min. and max. rest electron density −0.24, 0.28 eÅ^{-3}, number of variables 341.

*Methyl (3S)-3-{(3S)-3-{(3S)-3-[(*tert*-Butoxy)carbonylamino]-4-methylpentanoylamino}butanoylamino}-5-methylhexanoate* (Boc-β-*HVal*-β-HAla-β-HLeu-OMe; **1**; $C_{23}H_{43}N_3O_6$). Determination of the cell parameters and collection of the reflection intensities were performed on an *Enraf-Nonius-CAD4* four-circle diffractometer (graphite monochromatized CuK_α radiation, λ = 1.5418 Å). Colorless platelet, 0.1 × 0.3 × 0.7 mm, monoclinic, space group $P2_1$, a = 23.441 (3) Å, b = 5.020 (2) Å, c = 24.716 (2) Å, β = 109.251 (9)°, V = 2745.1 (12) Å^3, Z = 4, $\rho_{calc.}$ = 1.107 gcm^{-3}, μ = 0.647 mm^{-1}, $F(000)$ = 1000. Number of reflections measured 4607 ($\omega/2\theta$ scan, $2 < 2\theta < 50°$, T = 295 K); 4604 unique reflections, of which 2792 with $I > 2\sigma(I)$ were used for the determination (direct methods, SHELXS-86). SHELXL-93 was used for structure refinement (full-matrix least-squares). The non-H-atoms were refined anisotropically, the H-atoms were added to the molecule with constant isotropic temp. factors on idealized positions and refined according to the riding model (afix 3). The refinement converged at R = 0.074 (wR^2 = 0.194), min. and max. rest electron density −0.32, 0.35 eÅ^{-3}, number of variables 577.

REFERENCES

[1] H.-M. Müller, D. Seebach, *Angew. Chem.* **1993,** *105,* 483; *ibid. Int. Ed.* **1993,** *32,* 477; D. Seebach, A. Brunner, B. M. Bachmann, T. Hoffmann, F. N. M. Kühnle, U. D. Lengweiler, *Ernst Schering Research Foundation* **1995,** *28,* 7.

[2] A. F. Spatola, in 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins', Ed. B. Weinstein, Marcel Dekker, New York, 1983, Vol. 7, p. 267; C. N. C. Drey, in 'Chemistry and Biochemistry of Amino Acids', Ed. G. C. Barnet, Chapman and Hall, London, 1985, Chapt. 3, p. 25; D. F. Rane, V. M. Girijavallabhan, A. K. Ganguly, R. E. Pike, A. K. Saksena, A. T. McPhail, *Tetrahedron Lett.* **1993,** *34,* 3201; H. Onuki, K. Tachibana, N. Fusetani, *ibid.* **1993,** *34,* 5609; L. Yang, A. E. Weber, W. J. Greenlee, A. A. Patchett, *ibid.* **1993,** *34,* 7035.

[3] S. Shinagawa, T. Kanamaru, S. Harada, M. Asai, H. Okazaki, *J. Med. Chem.* **1987,** *30,* 1458; T. Oki, M. Hirano, K. Tomatsu, K.-I. Numata, H. Kamei, *J. Antibiot.* **1989,** *42,* 1756; G. Casiraghi, L. Colombo, G. Rassu, P. Spanu, *J. Org. Chem.* **1991,** *56,* 6523.

[4] G. L. Helms, R. E. Moore, W. P. Niemczura, G. M. L. Patterson, K. B. Tomer, M. L. Gross, *J. Org. Chem.* **1988,** *53,* 1298; S. M. Hecht, *Acc. Chem. Res.* **1986,** *19,* 383; K. S. Chu, G. R. Negrete, J. P. Konopelski, *J. Org. Chem.* **1991,** *56,* 5196; H. Sone, T. Nemoto, H. Ishiwata, M. Ojika, K. Yamada, *Tetrahedron Lett.* **1993,** *34,* 8449.

[5] R. M. J. Liskamp, *Recl. Trav. Chim. Pays-Bas* **1994,** *113,* 1.

[6] a) M. A. Ondetti, J. Pluscec, E. R. Weaver, N. Williams, E. F. Sabo, O. Kocy, in 'Chemistry and Biology of Peptides', Ann Arbor Science Publishers, Ann Arbor, 1972, p. 525; b) M. A. Ondetti, S. L. Engel, *J. Med. Chem.* **1975,** *18,* 761; c) O. W. Griffith, *Ann. Rev. Biochem.* **1986,** *55,* 855; d) K. Iizuka, T. Kamijo, H. Harada,

K. Akahane, T. Kubota, H. Umeyawa, Y. Kiso, *J. Chem. Soc., Chem. Commun.* **1989,** 1678; e) M. Rodriguez, P. Fulcrand, J. Laur, A. Aumelas, J.P. Bali, J. Martinez, *J. Med. Chem.* **1989,** *32,* 522; f) K. Stachowiak, M.C. Khosla, K. Plucińska, P.A. Khairallah, F.M. Bumpus, *ibid.* **1979,** *22,* 1128.

[7] W. Dürckheimer, J. Blumbach, R. Lattrell, K.H. Scheunemann, *Angew. Chem.* **1985,** *97,* 183; *ibid. Int. Ed.* **1985,** *24,* 180; D.J. Hart, D.-C. Ha, *Chem. Rev.* **1989,** *89,* 1447; W.A. Craig, S.C. Ebert, *Antimicrob. Agents Chemother.* **1992,** *36,* 2577; W.-B. Wang, E.J. Roskamp, *J. Am. Chem. Soc.* **1993,** *115,* 9417; 'The Organic Chemistry of β-Lactams', Ed. G.I. Georg, Verlag Chemie, NewYork, 1993.

[8] a) E. Juaristi, D. Quintana, J. Escalante, *Aldrichim. Acta* **1994,** *27,* 3; b) D.C. Cole, *Tetrahedron* **1994,** *50,* 9517; c) 'Enantioselective Synthesis of β-Amino Acids', Ed. E. Juaristi, VCH, New York, expected to appear at the end of 1996.

[9] a) F. Arndt, B. Eistert, W. Partale, *Ber. Dtsch. Chem. Ges.* **1927,** *60,* 1364; b) K. Balenović, *Experientia* **1947,** 369; c) M.S. Newman, P.F. Beal, *J. Am. Chem. Soc.* **1950,** *72,* 5163; d) D.S. Tarbell, J.A. Price, *J. Org. Chem.* **1957,** *22,* 245; e) B. Penke, J. Czombus, L. Baláspiri, J. Petres, K. Kovács, *Helv. Chim. Acta* **1970,** *53,* 1057; f) T. Ye, M.A. McKervey, *Chem. Rev.* **1994,** *94,* 1091; g) J.L. Matthews, C. Braun, C. Guibourdenche, M. Overhand, D. Seebach, in [8c], Chapt. 5.

[10] a) D. Fleš, A. Markovac-Prpic, *Croat. Chem. Acta* **1956,** *28,* 73; b) W. Jugelt, P. Falck, *J. Prakt. Chem.* **1968,** *38,* 88; c) H.M.M. Bastiaans, A.E. Alewijnse, J.L. van der Baan, H.C.J. Ottenheijm, *Tetrahedron Lett.* **1994,** *35,* 7659; d) J. Podlech, D. Seebach, *Angew. Chem.* **1995,** *107,* 507; *ibid. Int. Ed.* **1995,** *34,* 471; e) J. Podlech, D. Seebach, *Liebigs Ann. Chem.* **1995,** 1217; f) C. Guibourdenche, J. Podlech, D. Seebach, *ibid.*, submitted.

[11] a) I.L. Karle, P. Balaram, *Biochemistry* **1990,** *29,* 6747; b) I.L. Karle, J.L. Flippen-Anderson, K. Uma, P. Balaram, *Curr. Sci.* **1990,** *59,* 875; c) I.L. Karle, J.L. Flippen-Anderson, M. Sukumar, K. Uma, P. Balaram, *J. Am. Chem. Soc.* **1991,** *113,* 3952; d) D. Seebach, A. Studer, E. Pfammatter, H. Widmer, *Helv. Chim. Acta* **1994,** *77,* 2035.

[12] a) M. Goodman, C. Toniolo, F. Maider, in 'Peptides, Polypeptides and Proteins', Eds. E.R. Blout, F.A. Bovey, M. Goodman, and N. Loran, Wiley, New York, 1974, p. 308; b) C. Toniolo, G.M. Bonora, in 'Peptides: Chemistry, Structure and Biology', Eds. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, 1975, p. 145; c) K. Wüthrich, 'NMR of Proteins and Nucleic Acids', Wiley, New York, 1986; d) M.P. Williamson, J.P. Waltho, *Chem. Soc. Rev.* **1992,** *21,* 227.

[13] T.E. Creighton, *J. Phys. Chem.* **1985,** *89,* 2452.

[14] I.L. Karle, J.L. Flippen-Anderson, K. Ulma, M. Sukumar, P. Balaram, *J. Am. Chem. Soc.* **1990,** *112,* 9350.

[15] G.P. Dado, S.H. Gellman, *J. Am. Chem. Soc.* **1994,** *116,* 1054.

[16] a) R. Graf, G. Lohaus, K. Börner, E. Schmidt, H. Bestian, *Angew. Chem.* **1962,** *74,* 523; *ibid. Int. Ed.* **1962,** *1,* 481; b) H. Bestian, *Angew. Chem.* **1968,** *80,* 304; *ibid. Int. Ed.* **1968,** *7,* 278; c) E. Schmidt, *Angew. Makromol. Chem.* **1970,** *14,* 185; d) F. Chen, G. Lepore, M. Goodman, *Macromolecules* **1974,** *7,* 779; e) S.R. Turner, R.C. Schulz, *Makromol. Chem.* **1975,** *176,* 501; f) H.W. Siesler, *Polymer* **1974,** *15,* 146; g) H.R. Kricheldorf, G. Schilling, *Makromol. Chem.* **1978,** *179,* 2667; h) E.J. Günster, R.C. Schulz, *ibid.* **1980,** *181,* 643; i) J. Masamoto, K. Sasaguri, Ch. Ohizumi, H. Kobayashi, *J. Polym. Sci., Part A-2* **1970,** *8,* 1703; j) H. Yuki, Y. Okamoto, Y. Taketani, T. Tsubota, Y. Marubayashi, *J. Polym. Sci. Polym. Chem. Ed.* **1978,** *16,* 2237; k) J.M. Fernández-Santín, J. Aymamí, A. Rodríguez-Galán, S. Muñoz-Guerra, J.A. Subirana, *Nature (London)* **1984,** *311,* 53; l) J.M. Fernández-Santín, S. Muñoz-Guerra, A. Rodríguez-Galán, J. Aymami, J. Lloveras, J.A. Subirana, E. Giralt, M. Ptak, *Macromolecules* **1987,** *20,* 62; m) J. Kovacs, R. Ballina, R.L. Rodi, D. Balasubramanian, J. Applequist, *J. Am. Chem. Soc.* **1965,** *87,* 119.

[17] a) J. Lowbridge, C.N.C. Drey, *J. Chem. Soc., Chem. Commun.* **1970,** 791; b) C.N.C. Drey, J. Lowbridge, R.J. Ridge, *J. Chem. Soc., Perkin Trans. 1* **1973,** 2001; c) C.N.C. Drey, R.J. Ridge, *ibid.* **1981,** 2468; d) C.N.C. Drey, E. Mtetwa, *ibid.* **1982,** 1587; e) J. Lowbridge, E. Mtetwa, R.J. Ridge, C.N.C. Drey, *ibid.* **1986,** 155; f) D.N.J. White, C. Morrow, P.J. Cox, C.N.C. Drey, J. Lowbridge, *J. Chem. Soc., Perkin Trans. 2* **1982,** 239; g) M. Kajtár, M. Hollósi, Zs. Riedl, *Acta Chim. Acad. Sci. Hung.* **1976,** *88,* 301.

[18] L. Alig, A. Edenhofer, M. Müller, A. Trzeciak, T. Weller, Eur. Pat. Appl. EP 445796 A2 910911, 1991.

[19] H. Meier, K.-P. Zeller, *Angew. Chem.* **1975,** *87,* 52; *ibid. Int. Ed.* **1975,** *14,* 32.

[20] Y. Yukawa, Y. Tsuno, T. Ibata, *Bull. Chem. Soc. Jpn.* **1967,** *40,* 2613, 2618.

[21] J.C. Sheehan, P.A. Cruickshank, G.L. Boshart, *J. Org. Chem.* **1961,** *26,* 2525; N.L. Benoiton, K. Kuroda, F.M.F. Chen, *Int. J. Pept. Protein Res.* **1979,** *13,* 403.

[22] W. Steglich, G. Höfle, *Angew. Chem.* **1968,** *80,* 78; *ibid. Int.Ed.* **1968,** *7,* 61; W. Steglich, G.Höfle, *Chem. Ber.* **1969,** *102,* 883, 899; W. Steglich, G. Höfle, *Tetrahedron Lett.* **1970,** *11,* 4727.

[23] M. Bodanszky, Y.S. Klausner, M.A. Ondetti, 'Peptide Synthesis', 2nd edn., Wiley-Interscience, New York, 1976, p. 137; N.L. Benoiton, F.M.F. Chen, *Can. J. Chem.* **1981,** *59,* 384.

[24] S. Abdalla, E. Bayer, H. Frank, *Chromatographia* **1987,** *23,* 83.
[25] a) U. Schmidt, *Pure Appl. Chem.* **1986,** *58,* 295; b) L. Tomasic, G. P. Lorenzi, *Helv. Chim. Acta* **1987,** *70,* 1012; c) M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature (London)* **1993,** *366,* 324; d) M. R. Ghadiri, J. R. Granja, L. K. Buehler, *ibid.* **1994,** *369,* 301; e) J. R. Granja, M. R. Ghadiri, *J. Am. Chem. Soc.* **1994,** *116,* 10785; f) M. R. Ghadiri, K. Kobayashi, J. R. Granja, R. K. Chadha, D. E. McRee, *Angew. Chem.* **1995,** *107,* 76; *ibid. Int. Ed.* **1995,** *34,* 93; g) J. D. Hartgerink, J. R. Granja, R. A. Milligan, M. R. Ghadiri, *J. Am. Chem. Soc.* **1996,** *118,* 43.
[26] 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.
[27] a) Y.-H. Chen, J. T. Yang, H. M. Martinez, *Biochemistry* **1972,** *11,* 4120; b) C. Toniolo, G. M. Bonora, M. Mutter, *J. Am. Chem. Soc.* **1979,** *101,* 450; c) W. C. Johnson, Jr., *Proteins: Struct. Funct. Genet.* **1990,** *7,* 205.
[28] D. Seebach, H.-M. Müller, H. M. Bürger, D. A. Plattner, *Angew. Chem.* **1992,** *104,* 443; *ibid. Int. Ed.* **1992,** *31,* 434.
[29] D. A. Plattner, A. Brunner, M. Dobler, H.-M. Müller, W. Petter, P. Zbinden, D. Seebach, *Helv. Chim. Acta* **1993,** *76,* 2004; D. Seebach, T. Hoffmann, F. N. M. Kühnle, U. D. Lengweiler, *ibid.* **1994,** *77,* 2007.
[30] V. Tereshko, J. M. Monserrat, J. Pérez-Folch, J. Aymami, I. Fita, J. A. Subirana, *Acta Crystallogr., Sect. C* **1994,** *50,* 243.
[31] G. A. Jeffrey, W. Saenger, 'Hydrogen Bonding in Biological Structures', Springer Verlag, Berlin, 1991; J. P. Glusker, M. Lewis, M. Rossi, 'Crystal Structure Analysis for Chemists and Biologists', VCH, Weinheim, 1994.
[32] A. A. Bothner-By, R. L. Stephens, J. M. Lee, C. D. Warren, J. W. Jeanloz, *J. Am. Chem. Soc.* **1984,** *106,* 811; J. K. M. Sanders, B. K. Hunter, 'Modern NMR Spectroscopy, A. Guide for Chemists', Oxford University Press, Oxford, 1993.
[33] A. T. Brünger, in 'X-PLOR Manual V3.0', Yale University, New Haven, 1992.
[34] A. Widmer, personal communication.
[35] A. P. Ryle, in 'Methods in Enzymology', Eds. G. E. Perlman and L. Lorand, Academic Press, Orlando, 1970, Vol. 19, p. 316.
[36] J. S. Fruton, *Adv. Enzymol. Relat. Areas Mol. Biol.* **1976,** *44,* 1.
[37] P. Lombardi, *Chem. Ind. (London)* **1990,** (5 Nov.), 708; S. Moss, *ibid.* **1994,** (21 Feb.), 122.
[38] U. D. Lengweiler, M. G. Fritz, D. Seebach, *Helv. Chim. Acta.* **1996,** *79,* 670.
[39] M. Rothe, R. Theysohn, D. Mülhausen, F. Eisenbeiss, W. Schindler, in 'Chemistry and Biology of Peptides', Ed. J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, 1972, p. 51; E. R. Blout, *Biopolymers* **1981,** *20,* 1901; U. Schmidt, *Nachr. Chem. Tech. Lab.* **1989,** *37,* 1034.
[40] K. R. Shoemaker, P. S. Kim, E. J. York, J. M. Stewart, R. L. Baldwin, *Nature (London)* **1987,** *326,* 563; P. C. Lyu, M. I. Liff, L. A. Marky, N. R. Kallenbach, *Science* **1990,** *250,* 669; S. Marqusee, R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **1987,** *84,* 8898; K. T. O'Neil, W. F. DeGrado, *Science* **1990,** *250,* 646; C. Broger, K. Müller, in 'Structure Correlation', Eds. H.-B. Bürgi and J. D. Dunitz, VCH, Weinheim, 1994, p. 686; J. M. Scholtz, R. L. Baldwin, in 'Peptides, Synthesis, Structures, and Applications', Ed. B. Gutte, Academic Press, San Diego, 1995, p. 171.
[41] W. Kabsch, C. Sander, *Biopolymers* **1983,** *22,* 2577; G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985,** *37,* 1; J. S. Richardson, D. C. Richardson, in 'Prediction of Protein Structure and the Principles of Protein Conformation', Ed. G. D. Fasman, Plenum Press, New York, 1989.
[42] C. Toniolo, M. Crisma, G. M. Bonora, B. Klajc, F. Lelj, P. Grimaldi, A. Rosa, S. Polinelli, W. H. J. Boesten, E. M. Meijer, H. E. Schoemaker, J. Kamphuis, *Int. J. Pept. Protein Res.* **1991,** *38,* 242.
[43] G. Quinkert, E. Egert, C. Griesinger, 'Aspekte der Organischen Chemie', 'Struktur', Verlag Helvetica Chimica Acta, Basel, 1995, p. 125.
[44] a) R. L. Johnson, K. Verschoor, *J. Med. Chem.* **1983,** *26,* 1457; b) E. M. Gordon, J. D. Godfrey, N. G. Delaney, M. M. Asaad, D. Von Langen, D. W. Cushman, *ibid.* **1988,** *31,* 2199.
[45] R. Schwyzer, A. Costopanagiotis, P. Sieber, *Helv. Chim. Acta* **1963,** *46,* 87.
[46] M. Bodanszky, A. Bodanszky, 'The Practice of Peptide Synthesis', Springer-Verlag, New York, 1984, p. 177.
[47] U. Schmidt, H. Griesser, A. Lieberknecht, J. Talbiersky, *Angew. Chem.* **1981,** *93,* 271; *ibid. Int. Ed.* **1981,** *20,* 280.