γ-Peptides Forming More Stable Secondary Structures than α-Peptides: Synthesis and Helical NMR-Solution Structure of the γ-Hexapeptide Analog of H-(Val-Ala-Leu)$_2$-OH

by **Tobias Hintermann**[1]), **Karl Gademann**[1]), **Bernhard Jaun**, and **Dieter Seebach***

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

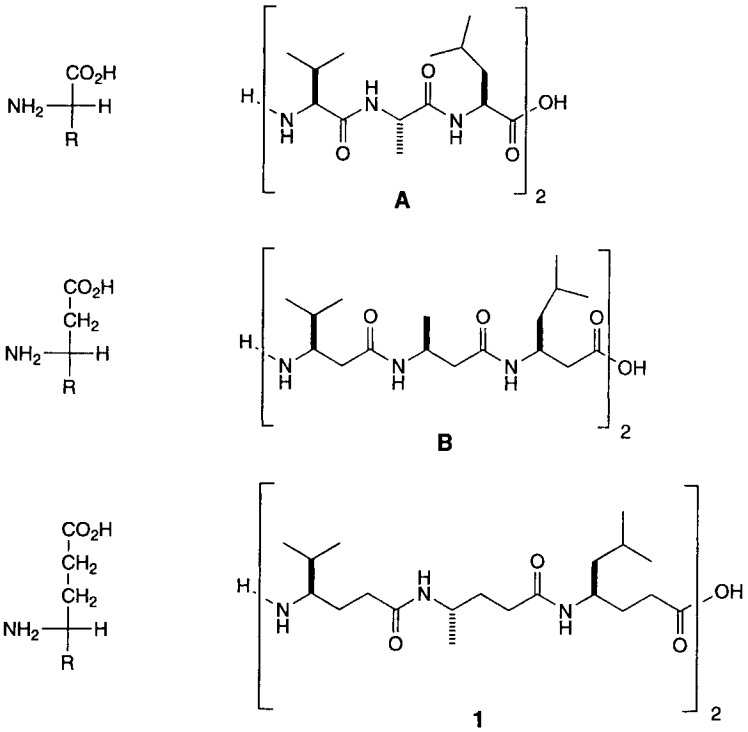
Dedicated to Professor *E. J. Corey* on the occasion of his 70th birthday

For a comparison with the corresponding α- and β-hexapeptides H-(Val-Ala-Leu)$_2$-OH (**A**) and H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**B**), we have now prepared the corresponding γ-hexapeptide **1** built from the homochirally similar (*S*)-4-aminobutanoic acid, (*R*)-4-amino-5-methylhexanoic acid, and (*R*)-4-amino-6-methylheptanoic acid. The precursors were prepared either by double *Arndt-Eistert* homologation of the protected amino acids Boc-Val-OH, Boc-Ala-OH, and Boc-Leu-OH (*Schemes 1* and *2*), or by the superior route involving olefination/hydrogenation of the corresponding aldehydes (Boc-valinal, Boc-alaninal, and Boc-leucinal; *Scheme 3*). Conventional peptide-coupling methodology (EDC/HOBt) furnished the γ-hexapeptide **1** (through the intermediate γ-di- and γ-tripeptide derivatives **9**–**11**). Analysis of NMR measurements in (D_5)pyridine and CD_3OH solution (COSY, TOCSY, HSQC, HMBC, ROESY) reveals that the γ-hexapeptide **1** adopts a *right*-handed helical structure ((*P*)-*2.6*$_1$ helix of *ca.* 5-Å pitch, containing 14-membered H-bonded rings) which is to be compared with the *left*-handed helix of the corresponding β-peptide **B** ((*M*)-*3*$_1$ helix of 5-Å pitch, 14-membered H-bonded rings) and with the familiar *right*-handed, so-called α-helix of α-peptides ((*P*)-*3.6*$_1$ helix of 5.4-Å pitch, 13-membered rings). Like the helix sense, the helix dipole reverses when going from α- (N ⟶ C) to β- (C ⟶ N) to γ-peptides (N ⟶ C). The surprising difference between the natural α-, and the analogous β- and γ-peptides is that the helix stability increases upon homologation of the residues.

1. Introduction. – Short-chain α-peptides such as **A** are known not to form secondary structures in solution. To the surprise of many chemists, analogs such as the β-hexapeptide **B** consisting of β- rather than α-amino acids, adopt stable helical backbone conformations in MeOH [1]. Apparently, the additional CH_2 group in each amino-acid residue of **B** does not lead to a more extended, flatter conformational-energy hypersurface, but to more pronounced minima as compared to those of corresponding α-peptides; after all, to the conformational preferences of α-peptides (as presented in the *Ramachandran* diagram [2]) has been added another element of conformational order: the staggering of the ethane bonds in the β-amino-acid residues [3]. We wondered what the effect of yet another CH_2 group would be, and have synthesized the γ-hexapeptide **1**, with the side chains of the natural amino-acid residues Val, Ala, Leu, and with L-configuration on all six chirality centers[2]).

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

[2]) All amino acids in the sequences **A**, **B**, and **1** are *homochiral* (or, in *Lord Kelvin*'s original definition, *homochirally similar* [4]), although their sense of chirality (absolute configuration) in the *CIP* convention may be (*R*) or (*S*); see *Exper. Part.*



The synthesis of the γ-hexapeptide **1** was completed by one of us (*T.H.*) in July 1997, and it took until now to solve its solution structure by NMR analysis. In an independent effort, *Hanessian* and coworkers have also synthesized a γ-peptide and determined its structure[3]).

2. Preparation of the γ-Amino-Acid Building Blocks. – There are four general methods described in the literature for the synthesis of enantiomerically pure *N*-protected γ-amino acids from the corresponding *N*-protected α-amino acids: the double *Arndt-Eistert* homologation [5][6] of Z- or phthalyl-protected α-amino acids, the alkylation of diethyl malonate by substituted *N*-tosyl-aziridines prepared *in situ* from *N,O*-bis(toluene-4-sulfonates) of amino alcohols (derived from α-amino acids) [6], the olefinative homologation of α-amino aldehydes [7][8], and the reduction of the keto functionality of α-aminoacyl *Meldrum*'s acid, followed by thermal decarboxylative ring closure to a 5-substituted pyrrolidinone (γ-lactam), and basic hydrolysis to the γ-amino acid [9]. Hitherto, only the last two methods have been used for the preparation of Boc-protected γ-amino acids.

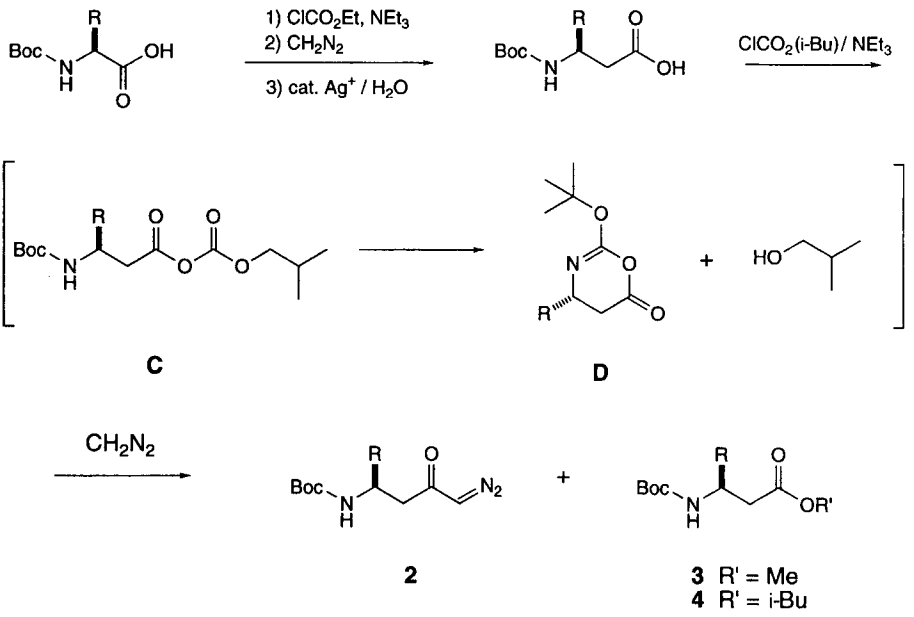
In our previous work [10][11], we have used the *Arndt-Eistert* homologation of α-amino acids for the preparation of enantiomerically pure β-amino-acid derivatives.

---

[3]) A letter from Prof. *S. Hanessian* (dated December 18, 1997), with figures showing the structures of a fully protected γ-tetra- and γ-hexapeptide was received by us on March 2, 1998. We gratefully acknowledge this personal communication.

With the Boc-protected β-amino acids at hand, we thought that their homologation would be a straightforward approach to enantiomerically pure Boc-protected γ-amino acids. In analogy with the homologation of α-amino acids, the *N*-Boc-protected β-amino acids were converted to mixed anhydrides **C** with $Et_3N/ClCO_2(i\text{-}Pr)$ and subsequently treated with excess CH_2N_2. However, applying the same reaction conditions as for the homologation of α-amino acids (3 h reaction time, room temperature), very low yields of the diazo ketones **2** were obtained (*Scheme 1*). Instead, the main product was, as revealed

Scheme 1



a R = Me; **b** R = Me_2CH; **c** R = Me_2CHCH_2

by ^{1}H-NMR spectroscopy, the heterocyclic imino anhydride **D**[4]), which appears to be much less reactive towards nucleophiles than the mixed anhydride **C**[5]). The use of a larger excess of CH_2N_2 (2 *vs.* 1.5 equiv.) and a prolonged reaction time (16 h at room temperature) led to complete conversion of the heterocycles **D** to a mixture of β-diazo ketones **2** (*ca.* 40–50%) and the β-amino acid esters **3** and **4** (20–25% each). This product distribution can be interpreted as resulting from rapid cyclization **C** → **D** and reaction of the liberated i-BuOH[6]) with **D**, in competition to CH_2N_2; the formation of

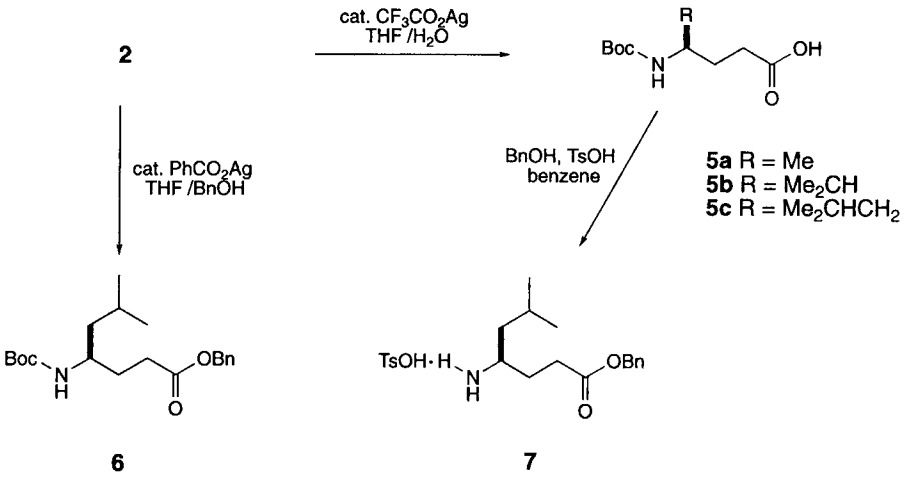
[4]) Such 4,5-dihydrooxazin-6-ones are also formed by cyclization of the intermediate ketenes from the *Wolff* rearrangement of amino-acid-derived α-diazo ketones in the absence of nucleophiles [12].

[5]) The cyclic anhydride **D** not only survived aqueous workup, but also, partially, flash chromatography on silica gel!

[6]) Formation of the mixed anhydride with $ClCO_2Et$ leads to a similar product distribution, containing the β-amino acid ethyl esters instead of the isobutyl esters.

methyl esters such as **3** is a well-known side reaction in the preparation of diazo ketones from activated carboxylic-acid derivatives and CH_2N_2 [10]. Whereas the separation of the diazo ketones **2** from the esters **3** and **4** was possible (FC and/or recrystallization, 41% of **2a**, 27% of **2b**, 37% of **2c**), the esters **3** and **4** could not be separated from each other[7][8]). The *Wolff* rearrangement of the β-diazo ketones **2** (*Scheme 2*), using the known procedures [10][11], gave the γ-amino acids **5** and, in the presence of benzyl alcohol (BnOH), the γ-amino acid ester **6** in good yields (66–67%). The benzyl ester hydrogen tosylate salt **7** was obtained from γ-amino acid **5c** by esterification.

Scheme 2



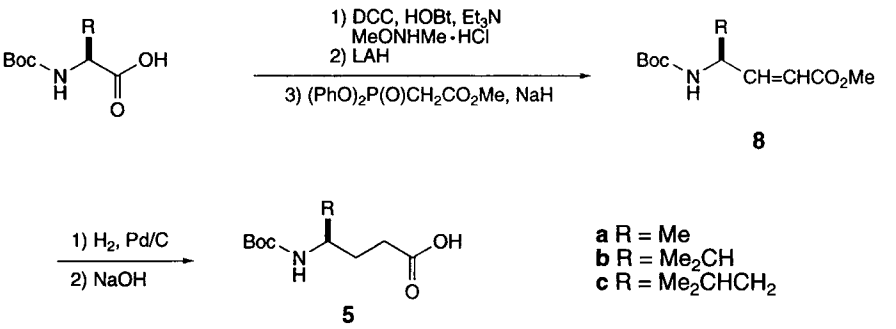
Since the formation of diazo ketones from Boc-protected β-amino acids turned out to be more difficult than expected, and since the use of CH_2N_2 in large quantities is not desirable, we decided to prepare the Boc-protected γ-amino acids by an olefination route (*Scheme 3*) [7][8], for which the single steps, but not their combination, were described in literature: The Boc-protected α-amino acids were converted to the *Weinreb* amides, and these were reduced to the α-amino aldehydes [13], which, in turn, were subjected to an olefination [14] to give the α,β-unsaturated Boc-protected γ-amino acid methyl esters **8** as *trans/cis* mixtures (3:1 to 7:1). Hydrogenation with Pd/C and saponification produced the desired Boc-protected γ-amino acids **5**. The overall yield of the five steps from Boc-protected α-amino acids was 55% for **5a**, 56% for **5b**, and 72% for **5c**, far superior to those obtained by double *Arndt-Eistert* homologation (< 20% over four steps from the same starting materials).

To determine the enantiomer purities of the γ-amino-acid building blocks thus prepared, we compared the optical rotations of Boc-protected compounds **5**, as prepared by the two independent routes, with literature data [9]. The optical activities of the

[7]) To prevent the loss of such large amounts of precious β-amino acids, the mixture of esters can be saponified to the starting materials.

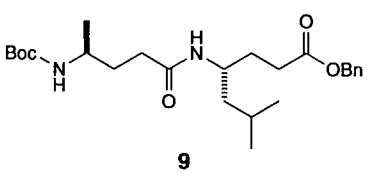
[8]) A possibility to prevent the formation of isobutyl esters **4** would be the use of an activation method (*e.g.*, acid-chloride formation [5]) that does not lead to a good nucleophile upon cyclization (**C** → **D**).

Scheme 3

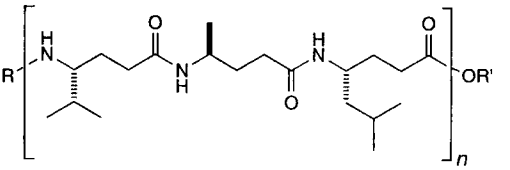


γ-amino acids prepared by the two routes were essentially identical, and they were higher than those obtained by the *Meldrum*-acid route [9].

3. Synthesis of the γ-Peptides. – We chose to use the same methodology as for our syntheses of β-peptides [3][15][16], *i.e.*, the fragment coupling with *N*-Boc and benzyl-ester protection[9]). Thus, the γ-amino acid hydrogen toluene-4-sulfonate **7** was employed for coupling with the Boc-protected γ-amino acid **5a**, using the standard method (NMM/EDC/HOBt [3])[10]). The γ-dipeptide derivative **9**, obtained in 95% yield, was Boc-deprotected (TFA/CH_2Cl_2) and coupled with the γ-amino acid **5b** to yield the γ-tripeptide derivative **10** (92% after FC). For the following fragment coupling step, part of **10** was deprotected on the C-terminus (hydrogenolysis over Pd/C in MeOH, → **11**), part on the N-terminus (HCl/dioxane), and the two γ-tripeptide units were coupled to give the protected γ-hexapeptide **12** in 73% yield. Whereas the analogous β-hexapeptide with the same sequence of side chains was very insoluble in any solvents, and, therefore, difficult to purify [15], the protected γ-hexapeptide **12** could easily be purified by FC (CH_2Cl_2/MeOH 20:1).



9



10 R = Boc, R' = Bn, *n* = 1
11 R = Boc, R' = H, *n* = 1
12 R = Boc, R' = Bn, *n* = 2
1 R = R' = H, *n* = 2, TFA salt

[9]) Like with β-substituted β-amino acids (and in contrast to the synthesis of α-peptides and of α-substituted β-peptides [3]), there are not epimerization problems to be expected in the coupling and deprotecting steps leading to γ-peptides.

[10]) Alternatively, the Boc-protected γ-amino acid benzyl ester **6** can be transformed to the hydrochloride or trifluoroacetate salt (by treatment with HCl/dioxane or with CF_3COOH (TFA)), and this can be used for peptide coupling.

For CD and NMR-spectroscopic investigations, unprotected peptides are preferred, in order to avoid possible effects of protecting groups on the secondary structure [3]. Therefore, the benzyl-ester group of **12** was hydrogenolytically cleaved (Pd/C, H_2, THF/AcOH 5:1)[11]) and the Boc-protection removed in TFA/CH_2Cl_2 1:1. The resulting crude γ-hexapeptide was purified by RP-HPLC to provide the TFA salt **1** in nearly quantitative yield.

CD Measurements with the free γ-hexapeptide **1** in MeOH (as well as with the protected γ-hexapeptide **12** and the *N*-protected γ-tripeptide **11**) showed no *Cotton* effect. Furthermore, the free γ-hexapeptide **1** has *no optical rotation* in MeOH, while the protected form **12** is optically active ($[\alpha]_D^{r.t.} = +\ 33.1$)[12]). Thus, in contrast to the situation with β-peptides (where a strong CD effect of **B** had been the first indication for the presence of a secondary structure [15]), there was no hint, whatsoever, from optical measurements as to whether the γ-peptide **1** has a distinct backbone conformation in solution. On the other hand, in the ^{1}H-NMR spectrum of **1**, the J(NH,H–C(γ)) values are rather large, indicating the presence of a secondary structure. We, therefore, decided to perform a detailed 2D-NMR analysis.

4. The Determination of the Solution Structure of 1 in (D_5)Pyridine by 2D-NMR Spectroscopy. – The assignment of all resonances in the ^{1}H-NMR spectrum as well as the determination of the sequence was achieved by DQF-COSY, TOCSY, HSQC, and HMBC experiments, and the chemical shifts are listed in *Table 1*. Diastereotopic

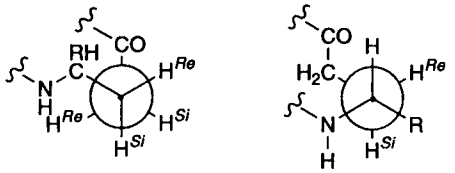
Table 1. *^{1}H-NMR Chemical Shifts* ((D_5)pyridine) *of the γ-Hexapeptide* **1**. Diastereotopic protons are labelled with primes in order of decreasing chemical shifts.

Residue	γ-HHVal[1]	γ-HHAla[2]	γ-HHLeu[3]	γ-HHVal[4]	γ-HHAla[5]	γ-HHLeu[6]
NH, NH_3	11.29 (br.)	8.94	8.39	8.11	8.15	8.27
H–C(γ)	3.62	4.29	4.44	4.20	4.48	4.43
H_{Re}–C(β)	2.37	2.22	2.34	2.41	2.32	2.11
H_{Si}–C(β)	2.14	1.77	1.64	1.70	1.96	1.94
H–C(α)[a])	2.99	2.77	2.81	2.76	2.75	2.74
H′–C(α)[b])	2.74	2.48	2.48	2.62	2.76	2.73
H–C(δ)	–	–	1.55	–	–	1.63
H′–C(δ)	–	–	1.39	–	–	1.37
H–C(δ)	2.24	–	–	1.82		–
CH_3(δ)	–	1.22	–	–	1.25	–
H–C(ε)	–	1.84	–	–	1.93	
CH_3(ε)	1.14/1.12	–	–	1.03/1.03	–	–
CH_3(ϕ)	–	–	1.01/0.95	–	–	1.01/0.96

[a]) H_{Re}–C(α) assigned for H–C(α) of residue 1 and H′–C(α) of residues 2, 3, and 4.
[b]) H_{Si}–C(α) assigned for H′–C(α) of residue 1 and H–C(α) of residues 2, 3, and 4.

[11]) The use of MeOH as solvent led to the formation of significant amounts of the hexapeptide methyl ester as a by-product.

[12]) The fully and half-protected γ-tripeptides **10** and **11**, respectively, are also optically active. Relatively low optical activities probably result from the fact that the γ-amino-acid building blocks **5a** and **5b** are dextro-, while **5c** is levorotatory.



C(β)—C(α) C(γ)—C(β)

Fig. 1. Newman *projections along the C(3)–C(2) (i.e., C(β)–C(α)) and C(4)–C(3) (i.e., C(γ)–C(β)) ethane bonds of the γ-amino-acid residues in the γ-hexapeptide* **1** *derived from a model of the solution structure.* Assignment of H_{Re}- and H_{Si}-atoms on the backbone CH_2 groups. Both conformations are (+)-*synclinal*.

$CH_2(\beta)$protons were assigned assuming that the small $^3J(H-C(\gamma),H-C(\beta))$ values in the COSY spectrum result from coupling to $H_{Re}-C(\beta)$ (*Fig. 1*). To gather information about the three-dimensional structure of γ-hexapeptide, a series of ROESY spectra at three different mixing times was acquired, and NOEs were extracted from the ROESY spectrum with a mixing time of 150 ms. These NOEs, which are summarized in *Table 2*, were classified according to their estimated cross-peak volume in the contour plot in three distance categories: strong, medium, and weak. A total of 42 NOEs was extracted, of which 20 NOEs are intra-residual, 13 sequential, 6 are from residue *i* to residue $(i + 2)$, and 3 from residue *i* to $(i + 3)$. These data were used as distance restraints in the simulated annealing protocol of X-PLOR 3.851 yielding 20 structures that could be clustered in a right-handed helix. H-Bond restraints were then added during the final refinement with a slow-cooling simulated annealing protocol. This gave the final bundle of 20 structures, depicted in *Fig. 2*, with a backbone RMSD of 0.3 Å for residues 2 to 5. We selected this bundle as representative for the structure in solution. It is very well-defined from residues 2 to 5, while the N-terminal residue, and, even more pronounced, the C-terminal residue are flexible. The side chains of residues γ-Leu 3 and γ-Val 4 are remarkably well-defined, whereas the side chains of the terminal residues are not.

The secondary structure of the γ-hexapeptide is a right-handed helix with H-bonds from C=O of residue *i* to NH of residue $i + 3$ and thus containing 14-membered H-bonding rings. This results in a dipole negative at the C-terminus and positive at the N-terminus. The helix has a pitch of *ca.* 5.0 Å and has *ca.* 2.6 residues per turn. From the top view (*Fig. 3*), it is evident that hydrophobic interactions may stabilize this helix, due to the juxtaposition of the side chains.

5. The Determination of the Solution Structure of 1 in CD_3OH by 2D-NMR Spectroscopy. – The assignment of all resonances in the ^1H-NMR spectrum as well as the determination of the sequence was again achieved by DQF-COSY, TOCSY, HSQC, and HMBC experiments, and the chemical shifts are given in *Table 3*. The dispersion of the chemical shifts of both pairs of diastereotopic CH_2 protons is much lower in CD_3OH compared to (D_5)pyridine (the aromaticity of the pyridine solvent increases the dispersion of the chemical shifts!).

Table 2. *Weak, Medium, and Strong NOEs Observed in the 150-ms ROESY Spectrum of the γ-Hexapeptide* **1** *in (D_5)Pyridine.* The number *i* indicates whether the NOE measured is intraresidual (0) or interresidual between residues *k* and *l* (at distance 1, 2, or 3).

Residue	Atom	Residue	Atom	Intensity	$i\ (k-l)$
2	NH	1	H_{Re}–C(α)	strong	1
2	NH	1	H_{Re}–C(β)	weak	1
2	NH	2	H_{Re}–C(β)	weak	0
2	NH	1	H_{Si}–C(β)	weak	1
2	NH	2	H_{Si}–C(β)	strong	0
3	NH	4	H_{Re}–C(α)	weak	1
3	NH	3	H_{Re}–C(β)	strong	0
3	NH	5	H_{Si}–C(β)	weak	2
3	NH	2	H_{Si}–C(β)	weak	1
3	NH	3	H_{Si}–C(β)	strong	0
3	NH	3	CH_2(δ)	strong	0
3	NH	2	H_{Re}–C(α)	strong	1
4	NH	3	H_{Re}–C(α)	strong	1
4	NH	4	H–C(δ)	strong	0
4	NH	4	H_{Si}–C(β)	strong	0
4	NH	2	H–C(γ)	strong	2
5	NH	4	H_{Re}–C(α)	strong	1
5	NH	4	H_{Re}–C(β)	weak	1
5	NH	5	H_{Re}–C(β)	medium	0
5	NH	2	H_{Re}–C(β)	weak	3
5	NH	5	H_{Si}–C(β)	strong	0
6	NH	3	H_{Re}–C(β)	medium	3
6	NH	6	H_{Re}–C(β)	medium	0
6	NH	3	CH_2(δ)	strong	3
6	NH	4	H–C(δ)	medium	2
1	H–C(γ)	1	H_{Re}–C(α)	weak	0
1	H–C(γ)	3	H_{Re}–C(α)	weak	2
1	H–C(γ)	1	H_{Re}–C(β)	strong	0
1	H–C(γ)	2	H_{Re}–C(β)	strong	1
1	H–C(γ)	1	H_{Si}–C(β)	medium	0
2	H–C(γ)	1	H_{Re}–C(α)	weak	1
2	H–C(γ)	4	H_{Re}–C(α)	medium	2
2	H–C(γ)	2	H_{Re}–C(α)	weak	0
2	H–C(γ)	2	H_{Re}–C(β)	strong	0
2	H–C(γ)	4	H_{Si}–C(β)	weak	2
3	H–C(γ)	3	H_{Re}–C(α)	medium	0
3	H–C(γ)	3	H–C(ε)	weak	0
4	H–C(γ)	3	H_{Re}–C(α)	weak	1
4	H–C(γ)	4	H_{Re}–C(β)	strong	0
4	H–C(γ)	4	H–C(δ)	strong	0
5	H–C(γ)	4	H_{Re}–C(α)	weak	1
5	H–C(γ)	5	H_{Re}–C(β)	strong	0

To determine the three-dimensional structure in CD_3OH, ROESY spectra at three different mixing times were acquired. However, the very small dispersion [13]) caused problems, since all CH_2(α) proton resonances are overlapping, and, therefore, NOEs

[13]) Note that the difference in chemical shifts between the diastereotopic CH_2 protons in CD_3OH is only in the range of up to 0.04 ppm.

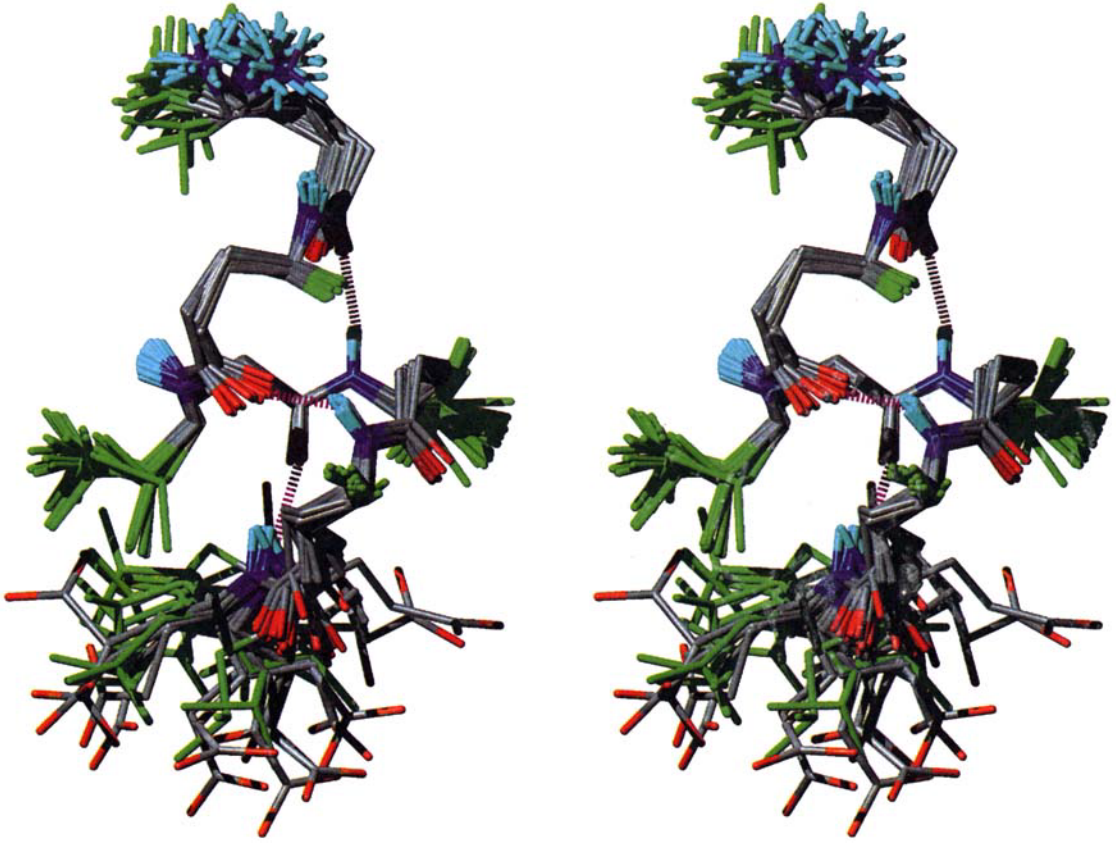


Fig. 2. *NMR-Solution structure* (stereo side view) *of the γ-hexapeptide* **1** *in* C_5D_5N. The γ-peptide forms a right-handed 2.6_{14} helix, as can be seen from the bundle of 20 conformers representative of the structure in solution. The side chains have been colored green, the backbone C-atoms are in grey, H-atoms in cyan, N-atoms in blue, and O-atoms in red. H-Bonds are marked with dashed lines in magenta. For clarity, all C-bound H-atoms have been omitted. The figure was generated by MolMol [17] and POV-Ray.

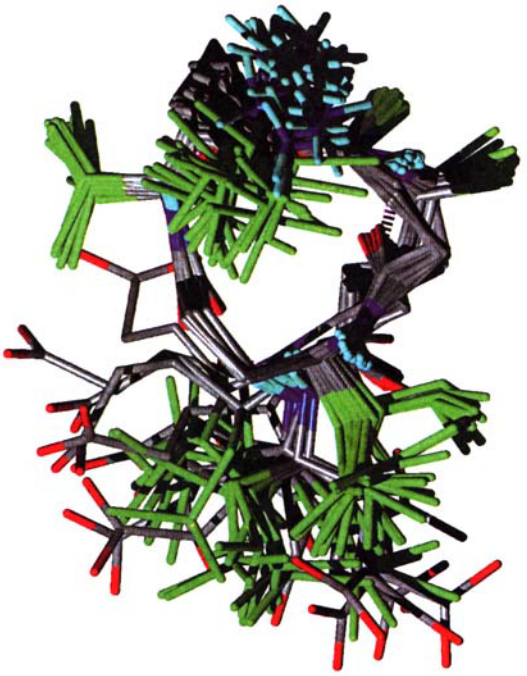


Fig. 3. *NMR-Solution structure* (top view) *of the γ-hexapeptide* **1** *in (D₅)pyridine.* It is evident from the top view that hydrophobic interactions may stabilize this helix (the side chains of valine, alanine, and leucine are in close juxtaposition). The coloring and the program for the representation are the same as in *Fig. 2.*

Table 3. *^{1}H-NMR Chemical Shifts* (CD_3OH) *of the γ-Hexapeptide* **1**. Diastereotopic protons are labelled with primes in order of decreasing chemical shifts.

Residue	γ-HHVal[1]	γ-HHAla[2]	γ-HHLeu[3]	γ-HHVal[4]	γ-HHAla[5]	γ-HHLeu[6]
NH, NH_3	n/a	7.90	7.56	7.54	7.87	7.81
H–C(γ)	2.96	3.80	3.89	3.55	3.83	3.86
H–C(β)	1.86	1.72	1.82	1.86	1.71	1.72
H′–C(β)	1.72	1.61	1.39	1.39	1.66	1.51
H–C(α)	2.33	2.17–2.13	2.17	2.13	2.17–2.13	2.26
H′–C(α)	2.29	2.17–2.13	2.15	2.13	2.17–2.13	2.24
H–C(δ)	–	–	1.28	–	–	1.3
H′–C(δ)	–	–	1.18	–	–	1.16
H–C(δ)	1.88	–	–	1.6	–	–
CH_3(δ)	–	1.04	–	–	0.82	–
H–C(ε)	–	–	1.52	–	–	1.55
CH_3(ε)	0.96/0.93	–	–	0.80/0.80	–	–
CH_3(φ)	–	–	0.82/0.80	–	–	0.83/0.81

from CH_2(α) to other protons could not be extracted. One region of the 150-ms ROESY spectrum, in which the cross-peaks are properly resolved, is the NH, H–C(γ) region (*Fig. 4*). This region contains valuable information, because the NOEs from H–C(γ) of residue *i* to the NH of residue (*i* + 2) are in perfect agreement with the structural bundle

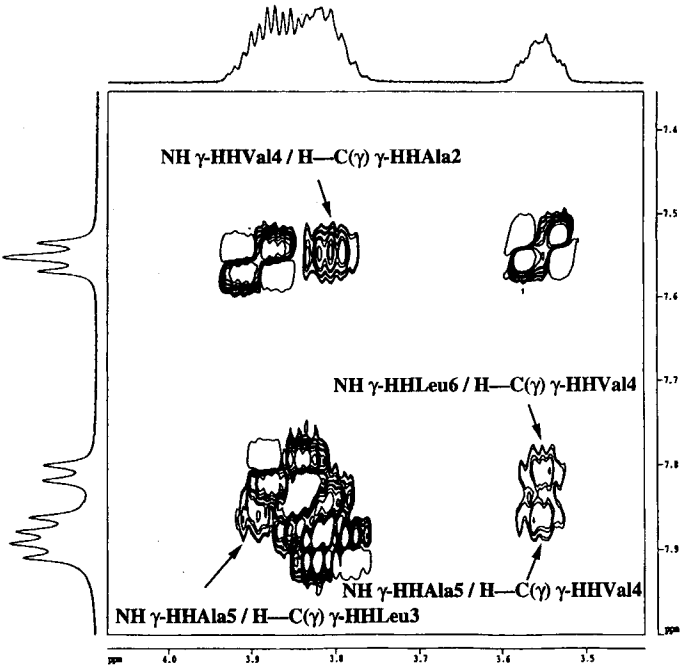


Fig. 4. *Part* (NH–C(γ) region) *of the 150-ms ROESY spectrum of* **1** *in* CD_3OH. The NOEs indicated by arrows offer strong evidence that the same helical structure as in C_5D_5N (*cf. Fig. 2*) is also populated in CD_3OH. Furthermore, these NOEs show that 14-membered H-bonds from residue *i* to (*i* + 3) are formed in CD_3OH solution.

depicted in *Fig. 2*, and these NOEs indicate that the same right-handed helical structure discovered in (D_5)pyridine is also populated in CD_3OH.

6. Discussion and Conclusions. – The discovery of a helical structure of a γ-hexapeptide in pyridine and methanol solution is a surprise, especially since no conformational restrictions such as rings[14]) or double bonds[15]) have been incorporated in the backbone of **1**. In a way, it is esthetically pleasing to find that insertion, in the α-carbonyl position, of one and two CH_2 groups, *i.e.*, single and double homologation, of the constituent amino acids with *retention of configuration*[16]) causes peptides to adopt helical structures of alternating opposite skrew senses and polarities[17]) (*Fig. 5*).

---

[14]) *Cf.* the β-peptides derived from *trans*-2-aminocyclopentane-[18] and -cyclohexanecarboxylic acids [19].

[15]) As in the vinylogous γ-oligopeptides composed of 4-aminoalk-2-enoic acids, derived from Ala, Val, and Phe [20].

[16]) For a suitable definition of *homologation with retention of configuration*, we refer to the nomenclature proposed by *Wintner* [21].

[17]) β-Peptide 3_1 helices, as the one shown in the center of *Fig. 5*, are favored by unprotected N-termini: apparently, the NH_3^+ charge stabilizes the dipolar structure with its negative pole at the N-terminus! This suggests that the γ-peptide helix, with its opposite N ⟷ C dipole, might actually be destabilized in the unprotected form (the structure of which we have determined), as compared to the protected form (see the investigation by *Hanessian et al.* mentioned in the *Introduction* and in *Footnote 3*).

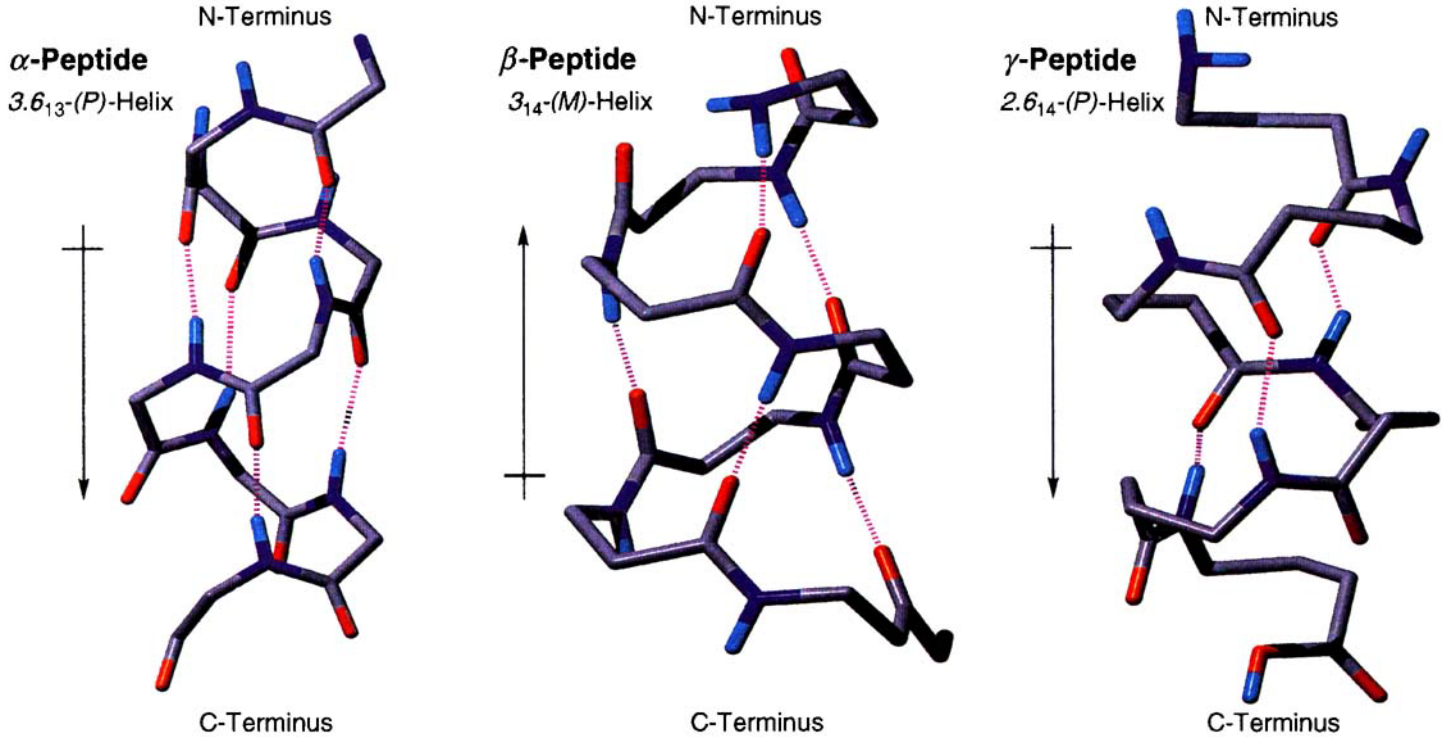


Fig. 5. *Models of helices of α-, β-, and γ-amino-acid oligomers.* The coordinates for the α-octapeptide (*left*) have been extracted from a protein X-ray structure [23]. The coordinates used for the β-heptapeptide (*center*) correspond to a solution structure examined previously by us [3]. The γ-hexapeptide (*right*) has been modelled from the backbone dihedral angles of the central leucine 3 residue. For clarity, the side chains as well as all C-bound H-atoms have been omitted in all three cases. The absolute configuration of the constituent α-, β-, and γ-amino acids generating the sense of helicity as shown is L in the *Fischer* convention (see *Formulae* **A**, **B**, **1**). The pitches of these α-, β-, and γ-peptide helices are 5.4, 5.0, and *ca.* 5 Å, respectively. The figure was generated by MolMol [17] and POV-Ray.

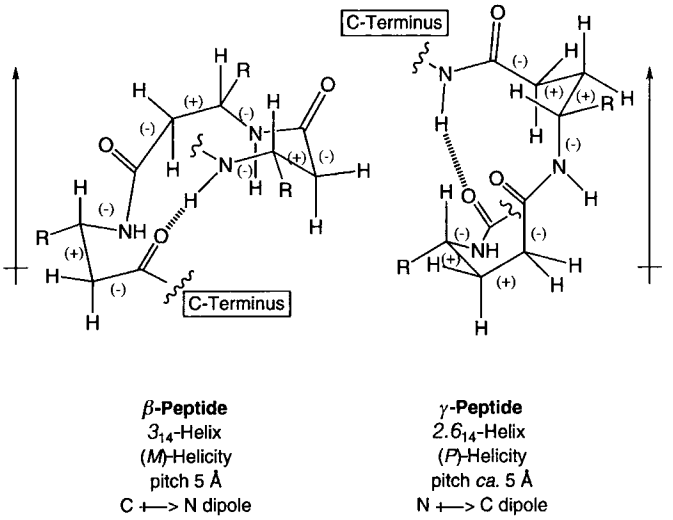


Fig. 6. *Schematic presentations of the 14-membered H-bonded rings present in a* 3_1 *helix of a β-peptide* [3] *and in the model of a γ-peptide helix shown in* Fig. 5. The signs (+) and (−) indicate the dihedral angles along intracatenate C,C and C,N bonds. Besides the β-peptide 3_1 helix shown here, two other helices of β-peptides have been identified in the past two years: a nonpolar (12/10/12)-helix, which appears to be favored by incorporation of the unsubstituted 3-aminopropanoic acid or by certain sequences of β^2- and β^3-amino acids [3], and a 2.5_{12} helix of opposite helicity and polarity (found in 2-aminocyclopentanecarboxylic-acid oligomers) [18].

A conformational analysis comparing the β- and the γ-peptide structures[18]) is presented in *Fig. 6*. The analysis reveals that, in order for a helix to be formed, it must carry the side chains in lateral positions (approximately perpendicular to the helix axis), the C=O and the N–H bonds, *i.e.*, the amide planes, must be approximately parallel to the helix axis, and the ethane bonds $CHR-CH_2$ and CH_2-CH_2 must be in a *synclinal* (*sc*) [22] conformation. With L-amino-acid-derived chirality centers, these ethane conformations have a (+)-*sc*-chirality. Thus, the reversal of helix sense and dipole direction upon going from β- to γ-peptides may be interpreted as a simple consequence of the additional CH_2 group and, thus, of a second (+)-*sc*-ethane moiety in the chains. The structural comparison in *Fig. 5* is somewhat unfair, because it does not include thermodynamic stability of the helices; a simple octapeptide from aliphatic amino acids *does not* form a stable secondary structure in solution, while the analogous β- and γ-peptides from six amino acids *do*.

It remains to be asked why the structures of β- and γ-peptides had not been discovered before 1995. This is not because there was no interest; polymers of β-amino acids (*Nylon-3*) have been made[19]) as early as 1962; this is also true of γ-peptides; linear [24][25] and cyclic [26] γ-oligo- and γ-polypeptides of the only proteinogenic γ-amino acid, glutamic acid[20]), have been reported in 1964, and so-called oligo(γ-glutamyl)

[18]) Everything has been said and written about the conformation of *α-peptides*; see textbooks and monographs on peptide and protein chemistry, and the excellent discussion in the book by *Quinkert et al.* [2].

[19]) See the list of references in our first paper [15] and in our recent feature article [1].

[20]) Simple γ-aminobutanoic-acid spacer peptides containing up to five residues of this achiral γ-amino acid have been incorporated in methotrexate analogs [27].

conjugates with pterine and with folates have been synthesized and investigated for pharmacological applications [25]. The reason for missing structural information about β- and γ-peptides was twofold: first of all, investigators stuck too strongly to their experiences about and knowledge of α-peptide secondary structures (when, for instance, trying to interpret CD spectra of β- and γ-oligopeptides), and more importantly, the powerful methods of 2D-NMR spectroscopy had not been available in the early days.

The authors would like to thank Ms. *B. Brandenberg* for recording some of the spectra. Financial support from *Novartis* is gratefully acknowledged.

Experimental Part

1. *General.* Abbreviations: BnOH (benzyl alcohol), DCC (1,3-dicyclohexylcarbodiimide), EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride), FC (flash chromatography), GP (general procedure), HOBt (1-hydroxy-1*H*-benzotriazole), h.v. (high vacuum, 0.01–0.1 Torr), β-HXxx (β-homoamino acid), γ-HHXxx (γ-homohomoamino acid), NMM (*N*-methylmorpholine). THF was freshly distilled over K under Ar before use. Et_3N was distilled over CaH_2 and stored over 4-Å molcular sieves. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). Amino-acid derivatives were purchased from *Senn Chemicals*. All other reagents were used as received from *Fluka*. The β-amino acids were prepared according to literature procedures [10][15][16]. ***Caution:*** The generation and handling of CH_2N_2 requires special precautions [28]. TLC: *Merck* silica gel *60* F_{254} plates; detection with UV or dipping into a soln. of anisaldehyde (9.2 ml), AcOH (3.75 ml), conc. H_2SO_4 (12.5 ml), and EtOH (338 ml), followed by heating. FC: *Fluka* silica gel *60* (40–63 μm); at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system (pump type *64*, *EuroChrom 2000* integration package, degaser, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz), *Varian Gemini 300* (^{1}H: 300 MHz, ^{13}C: 75 MHz), or *Gemini 200* (^{1}H: 200 MHz, ^{13}C: 50 MHz); chemical shifts (δ) in ppm downfield from Me_4Si (= 0 ppm); *J* values in Hz. MS: *VG Tribrid* (EI) or *Hitachi Perkin-Elmer RHU-6M* (FAB, in a 3-nitrobenzyl-alcohol matrix) spectrometer; in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Preparation of β-Diazo Ketones* **2.** *General Procedure 1* (*GP 1*). The Boc-protected β-amino acid (1 equiv.) was dissolved in THF (0.2M) under Ar and cooled to −25°. After addition of Et_3N (1 equiv.) and i-BuOCOCl (1 equiv.) the mixture was stirred at *ca.* −20° for 30 min. The resulting white suspension was warmed to 0° (ice-bath), and a soln. of CH_2N_2 (2.0 equiv.) in Et_2O was added. Stirring was continued for 16 h at r.t. Excess CH_2N_2 was destroyed by the addition of AcOH. The mixture was diluted with H_2O and the bulk of THF evaporated. After addition of Et_2O, the mixture was washed with 10%-aq. citric-acid soln., sat. K_2CO_3 soln., and sat. NaCl soln. The org. phase was dried (Na_2SO_4) and evaporated. FC and/or recrystallization afforded the pure diazo ketone.

3. *Preparation of* Weinreb *Amides. General Procedure 2* (*GP 2*). The Boc-protected α-amino acid (1 equiv.), HOBt (1 equiv.), and *N,O*-dimethylhydroxylamine hydrochloride were dissolved in CH_2Cl_2 (0.5M), the soln. was cooled (ice-bath), and Et_3N (2 equiv.) and DCC (1 equiv.) were added. The mixture was stirred at 0° for 1 h, then at r.t. for 4 h, and cooled again to 0°. The white precipitate (dicyclohexylurea) was filtered off and the filtrate washed with 1M NaOH soln. (2 ×), 10%-aq. citric-acid soln. (2 ×), and sat. NaCl soln. The org. phase was dried (Na_2SO_4) and evaporated. FC afforded the pure *Weinreb* amides.

4. *Preparation of α,β-Unsaturated γ-Amino Acids* **5.** *General Procedure 3* (*GP 3*) [13][14]. To a cooled (ice-bath) soln. of the *Weinreb* amide (1 equiv.) in Et_2O or THF (0.1M), $LiAlH_4$ (1.25 equiv.) was added. The mixture was stirred at r.t. for 20 min, then a soln. of $NaHSO_4$ (1.75 equiv.) in H_2O (0.33M) was added. The mixture was extracted with Et_2O (3 ×), the org. phases washed with 1M HCl soln. (3 ×), sat. $KHCO_3$ soln. (3 ×), and sat. NaCl soln., dried (Na_2SO_4), and evaporated. The resulting aldehyde was stored at −20° and used without further purification.

Trimethylphosphonoacetate (3 equiv.) was dissolved in THF (0.3M) and cooled (ice-bath). The addition of NaH (2 equiv.) led to a gelatinous mass which was stirred at r.t. for 30 min. The mixture was cooled again

(ice-bath), a soln. of the amino aldehyde (1 equiv.) in THF (0.17M) was added, the mixture was stirred for 5 min at 0° and for 20 min at r.t. The mixture was quenched with H_2O, and sat. NaCl soln. and AcOEt (approx. the same volume as THF each) were added, the org. phase was separated, dried (Na_2SO_4), and evaporated. The crude unsaturated γ-amino acid was purified by FC.

5. *Hydrogenation of α,β-Unsaturated γ-Amino Acids* **8** *and Hydrolysis of the Methyl Ester. General Procedure 4* (*GP 4*). To a soln. of the γ-amino acid methyl ester in MeOH (0.2M), 10% (*w*/*w*) of Pd/C (10%) was added. The apparatus was evacuated and flushed three times with H_2, and the mixture was stirred at r.t. for 20 h under H_2 (ballon). Filtration through *Celite* and concentration under reduced pressure yielded the crude methyl carboxylate which was used without further purification.

The Boc-protected amino ester (1 equiv.) was dissolved in MeOH (0.5M), and 1M NaOH soln. (2 equiv.) was added. The mixture was stirred at r.t. for 20 h, diluted with H_2O, and washed with Et_2O. The aq. phase was acidified with 6M HCl soln. (pH 1–2) and extracted with Et_2O (2×), the org. phases were washed with sat. NaCl soln., dried (Na_2SO_4), and evaporated. The crude product was recrystallized to yield pure Boc-protected γ-amino acids **5**.

6. *Peptide Coupling with EDC. General Procedure 5* (*GP 5*). The appropriate HCl, CF_3COOH, or TsOH salt was dissolved in THF. The Boc-protected fragment and HOBt were added, and the mixture was cooled to 0° (ice-bath) and successively treated with NMM and EDC. The soln. was stirred for 1 h at 0° then at r.t. for 18 h. The mixture was diluted with AcOEt and washed with 0.5M HCl (3×), sat. K_2CO_3 (3×) and sat. NaCl solns., the org. phase dried (Na_2SO_4) and concentrated under reduced pressure.

*(S)-1-Diazo-4-[(*tert*-butoxycarbonyl)amino]pentan-2-one* (**2a**). Boc-(*S*)-β^3-HAla-OH (5.08 g, 25 mmol) was transformed according to *GP 1*. FC (Et_2O/pentane 1:1, then 2:1) and recrystallization (Et_2O/pentane) yielded **2a** (2.31 g, 41%). Bright-yellow needles. M.p. 77–78°. $[\alpha]_D^{r.t.} = -18.0$ (*c* = 0.84, $CHCl_3$). IR ($CHCl_3$): 3441*m*, 3117*w*, 3007*m*, 2980*m*, 2110*s*, 1704*s*, 1636*m*, 1501*s*, 1456*m*, 1368*s*, 1331*m*, 1165*s*, 1099*m*, 1054*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.23 (*d*, *J* = 6.7, Me); 1.44 (*s*, Me_3C); 2.42–2.62 (br. *m*, CH_2); 3.98 (*m*, NCH); 4.99 (br. *s*, NH); 5.31 (*s*, CHN_2). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.6, 28.4 (Me); 44.3 (CH); 46.7 (CH_2), 55.4 (CH); 79.4, 155.2, 193.1 (C). EI-MS: 228 (< 1, $[M + H]^+$), 199 (3), 172 (8), 154 (28), 144 (53), 143 (69), 57 (100). Anal. calc. for $C_{10}H_{17}N_3O_3$ (227.26): C 52.85, H 7.54, N 18.49; found: C 52.94, H 7.40, N 18.49.

*(R)-1-Diazo-4-[(*tert*-butoxycarbonyl)amino]-5-methylhexan-2-one* (**2b**). Boc-(*R*)-β^3-HVal-OH (5.78 g, 25 mmol) was transformed according to *GP 1*. Recrystallization (AcOEt/pentane) yielded **2b** (1.27 g, 20%). Yellow needles. FC (Et_2O/pentane 1:1, then 2:1) of the mother liquor and recrystallization (Et_2O/pentane) yielded further **2b** (0.44 g, 7%). M.p. 104–106°. $[\alpha]_D^{r.t.} = -30.9$ (*c* = 0.95, $CHCl_3$). IR ($CHCl_3$): 3437*m*, 3116*w*, 3007*m*, 2968*m*, 2110*s*, 1703*s*, 1636*m*, 1501*s*, 1368*s*, 1166*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.8, 2 Me); 1.43 (*s*, Me_3C); 1.80–1.92 (*m*, CH); 2.52 (br. *m*, CH_2); 3.64–3.76 (*m*, NCH); 4.93 (br. *s*, NH); 5.38 (br. *s*, CHN_2). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.5, 19.5, 28.4 (Me); 31.9 (CH); 43.3 (CH_2); 53.7, 55.1 (CH); 79.2, 155.8, 193.5 (C). EI-MS: 256 (4, $[M + H]^+$), 212 (70), 200 (16), 184 (41), 182 (32), 172 (100), 156 (65). Anal. calc. for $C_{12}H_{21}N_3O_3$ (255.32): C 56.45, H 8.29, N 16.46; found: C 56.26, H 8.00, N 16.52.

*(S)-1-Diazo-4-[(*tert*-butoxycarbonyl)amino]-6-methylheptan-2-one* (**2c**). Boc-(*S*)-β^3-HLeu-OH (6.13 g, 25 mmol) was transformed according to *GP 1*. FC (Et_2O/pentane 1:1) yielded **2c** (2.50 g, 37%). Yellow solid. M.p. 83–85°. $[\alpha]_D^{r.t.} = -29.3$ (*c* = 1.29, $CHCl_3$). IR ($CHCl_3$): 3437*m*, 3116*w*, 3007*m*, 2961*m*, 2109*s*, 1703*s*, 1636*s*, 1503*s*, 1368*s*, 1165*s*, 1014*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*d*, *J* = 6.7, Me); 0.92 (*d*, *J* = 6.5, Me); 1.27–1.34 (*m*, CH); 1.40–1.52 (*m*, 1H, CH_2); 1.43 (*s*, Me_3C); 1.59–1.69 (*m*, 1H, CH_2); 2.51 (br. *m*, $C(O)CH_2$); 3.89–3.97 (*m*, NCH); 4.92 (br. *s*, NH); 5.33 (*s*, CHN_2). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.1, 23.0 (Me); 25.0 (CH); 28.4 (Me); 43.7, 45.7 (CH_2); 46.6, 55.3 (CH); 79.2, 155.4, 193.4 (C). EI-MS: 241 (3), 212(13), 196 (44), 186 (51), 185 (100), 128 (35), 84 (60), 57 (73). Anal. calc. for $C_{13}H_{23}N_3O_3$ (269.34): C 57.97, H 8.61, N 15.60; found: C 58.02, H 8.45, N 15.37.

*(S)-4-[(*tert*-Butoxycarbonyl)amino]pentanoic Acid* (**5a**). *a*) A soln. of **2a** (2.04 g, 9.0 mmol) in THF/H_2O (29 ml/3.2 ml) was cooled to −25°. A soln. of CF_3CO_2Ag (198 mg, 0.9 mmol) in Et_3N (3.2 ml) was added, the mixture slowly warmed to r.t. and stirred overnight. The mixture was diluted with Et_2O and extracted with 1M NaOH soln. The aq. phase was acidified with 6M HCl soln. extracted with Et_2O, dried (Na_2SO_4), and evaporated to give **5a** (1.34 g, 69%) as white solid which was used without further purification. $[\alpha]_D^{r.t.} = +2.3$ (*c* = 4.0, EtOH).

b) According to *GP 4* amino acid ester **8a** (3.51 g, 15.3 mmol) was transformed to **5a**. Recrystallization (pentane/Et_2O) gave **5a** (2.89 g, 87%). White needles. M.p. 83–84°. $[\alpha]_D^{r.t.} = +2.4$ (*c* = 4.0, EtOH). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.17 (*d*, *J* = 6.5, Me); 1.46 (*s*, Me_3C); 1.63–1.90 (*m*, CH_2); 2.42 (*t*, *J* = 7.0, $C(O)CH_2$); 3.61–3.83 (*m*, NCH); 4.40 (br. *s*, NH).

*(R)-4-[(*tert*-Butoxycarbonyl)amino]-5-methylhexanoic Acid* (**5b**). *a*) A soln. of **2b** (1.49 g, 5.8 mmol) in THF/H_2O (21.7 ml/2.3 ml) was cooled to −25°. A soln. of CF_3CO_2Ag (147 mg, 0.64 mmol) in Et_3N (2.4 ml) was

added, the mixture slowly warmed to r.t. and stirred overnight. The mixture was diluted with Et_2O and extracted with 1M NaOH soln. The aq. phase was acidified with 6M HCl soln., extracted with Et_2O (2 ×), washed with sat. NaCl soln., dried (Na_2SO_4), and evaporated to give **5b** (0.92 g, 65%) as white solid which was used without further purification. $[\alpha]_D^{r.t.} = +4.5$ ($c = 4.0$, EtOH).

b) According to *GP 4* amino acid ester **8b** (3.86 g, 15 mmol) was transformed to **5b**. Recrystallization (pentane/Et_2O) gave **5b** (3.13 g, 85%). White needles. M.p. 109–110° ([9]: 103–105°). $[\alpha]_D^{r.t.} = +4.0$ ($c = 4.0$, EtOH) ([9]: $[\alpha]_D^{r.t.} = +2.9$ ($c = 4.0$, EtOH)). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.7$, Me); 0.93 (*d*, $J = 6.7$, Me); 1.44 (*s*, Me_3C); 1.50–1.95 (*m*, Me_2CH, CH_2); 2.35–2.45 (*m*, $C(O)CH_2$); 3.30–3.52 (rotamers, *m*, CHN); 4.37, 5.73 (rotamers, *d*, $J = 9.8$, NH).

*(R)-4-[(*tert*-Butoxycarbonyl)amino]-6-methylheptanoic Acid* (**5c**). According to *GP 4* amino acid ester **8c** (5.02 g, 18.5 mmol) was transformed to **5c** (4.23 g, 88%). White solid. M.p. 108–110° ([9]: 109.5–111.5°). $[\alpha]_D^{r.t.} = -11.4$ ($c = 2.5$, EtOH) ([9]: $[\alpha]_D^{r.t.} = -10.7$ ($c = 2.9$, EtOH)). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.6$, 2 Me); 1.20–1.28 (*m*, Me_2CHCH_2); 1.43 (*s*, Me_3C); 1.52–1.73 (*m*, $CH_2CH_2C(O)$); 1.78–1.92 (*m*, Me_2CH); 2.33–2.46 (*m*, $C(O)CH_2$); 3.56–3.76 (br. *m*, NCH); 4.28, 5.41 (rotamers, *d*, $J = 9.0$, NH).

*Benzyl (R)-4-[(*tert*-Butoxycarbonyl)amino]-6-methylheptanoate* (**6**). A soln. of **2c** (2.16 g, 8.0 mmol) in THF/BnOH (24 ml/8 ml) was cooled to −25°. A soln. of $PhCO_2Ag$ (201 mg, 0.90 mmol) in Et_3N (3.2 ml) was added, the reaction mixture slowly warmed to r.t. and stirred for 7 h. The bulk of THF was evaporated, the residue diluted with Et_2O, filtered over *Celite* and SiO_2, washed with 10% aq. citric-acid soln. (2 ×), sat. K_2CO_3 soln. (2 ×), sat. NaCl soln., dried (Na_2SO_4), and evaporated. BnOH was removed under h.v. at 80° for 12 h. The resulting residue was recrystallized (Et_2O/pentane) to give **6** (1.88 g, 67%). White needles. M.p. 78–79°. $[\alpha]_D^{r.t.} = -10.2$ ($c = 1.02$, $CHCl_3$). IR ($CHCl_3$): 3436*m*, 3008*m*, 2960*m*, 1707*s*, 1503*s*, 1454*m*, 1392*m*, 1368*m*, 1169*s*, 1048*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.7$, 2 Me); 1.19–1.33 (*m*, CH_2); 1.42 (*s*, Me_3C); 1.58–1.68 (*m*, CH_2); 1.82–1.91 (*m*, CH); 2.37–2.49 (*m*, $C(O)CH_2$); 3.61–3.72 (*m*, CHN); 4.21 (*d*, $J = 9.1$, NH); $\nu_A = 5.11$, $\nu_B = 5.13$ (*AB*, $J = 12.4$, CH_2O); 7.29–7.38 (*m*, arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.3, 23.0 (Me); 24.9 (CH); 28.4 (Me); 31.0, 31.1, 45.2 (CH_2); 48.5 (CH); 66.3 (CH_2); 79.0 (C); 128.2, 128.2, 128.6 (CH); 136.0, 155.6, 173.5 (C). DEI-MS: 349 (< 1, M^+), 292 (5), 248 (19), 192 (44), 91 (100), Anal. calc. for $C_{20}H_{31}NO_4$ (349.47): C 68.74, H 8.94, N 4.01; found: C 68.74, H 8.99, N 3.98.

Benzyl (R)-Amino-6-methylheptanoate Hydrogen Toluene-4-sulfonate (**7**). A soln. of **5c** (4.23 g, 16.3 mmol) and TsOH · H_2O (3.72 g, 19.6 mmol) in benzene (125 ml) and BnOH (13 ml) was heated at reflux for 9 h, and H_2O formed in the reaction was trapped in a *Dean-Stark* receiver. The clear mixture was cooled to r.t., and pentane (100 ml) was added to yield **7** (2.71 g, 39%) as colorless needles. The mother liquor was evaporated to dryness and recrystallized from Et_2O to yield further **7** (3.79 g, 55%). Colorless needles. M.p. 137–140°. $[\alpha]_D^{r.t.} = +10.2$ ($c = 1.55$, $CHCl_3$). IR ($CHCl_3$): 3500–2400 (br.), 3007*s*, 2881*s*, 1731*s*, 1530*m*, 1455*m*, 1173*s*, 1124*s*, 1034*s*, 1010*s*. ^{1}H-NMR (400 MHz, CD_3OD): 0.91 (*d*, $J = 6.5$, Me); 0.92 (*d*, $J = 6.6$, Me); 1.44 (*t*, $J = 7.2$, $CH_2CH_2C(O)$); 1.65–1.75 (*m*, Me_2CH); 1.81–2.01 (*m*, Me_2CHCH_2); 2.35 (*s*, Me); 2.54 (*t*, $J = 7.4$, $CH_2C(O)$); 3.24–3.31 (*m*, CHN); 5.13 (*s*, CH_2O); 7.20–7.23 (*m*, 2 arom. H); 7.29–7.37 (*m*, 5 arom. H); 7.69–7.72 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 21.3, 22.5, 22.7 (Me); 25.4 (CH); 29.1, 30.5, 42.9 (CH_2); 50.6 (CH); 67.6 (CH_2); 127.0, 129.3, 129.4, 129.6, 129.8 (CH); 137.4, 141.7, 143.5, 173.9 (C): MS (ESI$^+$): 250 (100, $[M + H]^+$ of amino acid ester), 91(20). MS (ESI$^-$): 171 (100, $[M - H]^-$ of TsOH). Anal. calc. for $C_{22}H_{31}NO_5S$ (421.55): C 62.68, H 7.41, N 3.32; found: C 62.75, H 7.41, N 3.42.

*Methyl (S)-4-[(*tert*-Butoxycarbonyl)amino]pent-2-enoate* (**8a**). Boc-Ala-OH (9.46 g, 50 mmol) was treated according to *GP 2* to yield, after purification by FC (Et_2O), the *Weinreb* amide (9.00 g, 82%) as white solid. Treatment of this *Weinreb* amide (4.41 g, 20 mmol) in THF according to *GP 3* led, after FC (pentane/Et_2O 3:1), to **8a** (3.51 g, 77%, (*E*)/(*Z*)-mixture (4.9:1)). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$, (*E*)-isomer): 1.28 (*d*, $J = 6.0$, Me); 1.46 (*s*, Me_3C); 3.76 (*s*, MeO); 4.41 (br. *m*, CHN); 4.52 (br. *s*, NH); 5.73 (*d*, $J = 15.5$, CH); 6.90 (*dd*, $J_1 = 15.5$, $J_2 = 5.0$, CH).

*Methyl (S)-4-[(*tert*-Butoxycarbonyl)amino]-5-methylhex-2-enoate* (**8b**). Boc-Val-OH (10.9 g, 50 mmol) was treated according to *GP 2* to yield, after purification by FC (Et_2O/pentane 1:1), the *Weinreb* amide (11.3 g, 87%) as colorless oil. Treatment of this *Weinreb* amide (5.21 g, 20 mmol) in Et_2O according to *GP 3* led, after FC (pentane/Et_2O 3:1), to **8b** (3.89 g, 76%, (*E*)/(*Z*)-mixture (7.3:1)). White solid. ^{1}H-NMR (200 MHz, $CDCl_3$, (*E*)-isomer): 0.93 (*t*, $J = 6.0$, 2 Me); 1.45 (*s*, Me_3C); 1.76–1.96 (*m*, Me_2CH); 3.73 (*s*, MeO); 4.27 (br. *m*, CHN); 4.55 (br. *s*, NH); 5.93 (*d*, $J = 15.5$, CH); 6.87 (*dd*, $J_1 = 15.5$, $J_2 = 5.0$, CH).

*Methyl (S)-4-[(*tert*-Butoxycarbonyl)amino]-6-methylhept-2-enoate* (**8c**). Boc-Leu-OH (12.5 g, 50 mmol) was treated according to *GP 2* to yield, after purification by FC (Et_2O), the *Weinreb* amide (12.3 g, 89%) as colorless oil. Treatment of this *Weinreb* amide (5.49 g, 20 mmol) in Et_2O according to *GP 3* led, after FC (pentane/Et_2O 3:1), to **8c** (5.02 g, 92%, (*E*)/(*Z*)-mixture (2.5:1)). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$, (*E*)-isomer):

0.96 (*d*, J = 6.0, 2 Me); 1.35–1.45 (*m*, CH_2); 1.44 (*s*, Me_3C); 1.60–1.80 (*m*, Me_2CH); 3.75 (*s*, MeO); 4.27–4.42 (*m*, NCH); 4.44 (br. *s*, NH); 5.94 (*d*, J = 15.5, CH); 6.85 (*dd*, J_1 = 15.5, J_2 = 5.5, CH).

Boc-(S)-γ-HHAla-(R)-γ-HHLeu-OBn (**9**). Benzyl ester **7** (3.37 g, 8.0 mmol), **5a** (1.74 g, 8.0 mmol), HOBt (1.30 g, 9.6 mmol), NMM (2.16 ml, 19.2 mmol), and EDC (1.54 g, 8.0 mmol) in THF (40 ml) were treated according to *GP 5* to yield **9** (3.42 g, 95%) as white solid which was used without further purification. M.p. 113–114°. $[\alpha]_D^{r.t.} = -4.7$ (c = 0.81, $CHCl_3$). IR ($CHCl_3$): 3500–3200 (br.), 3435*m*, 2968*m*, 1730*m*, 1696*s*, 1663*m*, 1507*s*, 1454*m*, 1368*m*, 1169*s*, 1064*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, J = 6.5, Me); 0.90 (*d*, J = 6.7, Me); 1.11 (*d*, J = 6.6, Me); 1.21–1.29 (*m*, 1H, CH_2); 1.32–1.39 (*m*, 1H, CH_2); 1.43 (*s*, Me_3C); 1.56–1.79 (*m*, 2 CH_2); 1.85–1.93 (*m*, CH); 2.11–2.22 (*m*, $C(O)CH_2$); 2.36–2.49 (*m*, $C(O)CH_2$); 3.57–3.70 (br. *m*, CHN); 3.97–4.06 (*m*, CHN); 4.48 (br. *d*, CO_2NH); 5.11 (*s*, CH_2O); 6.11 (br. *d*, C(O)NH); 7.29–7.38 (*m*, arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.5, 22.3, 23.1 (Me); 25.0 (CH); 28.4 (Me); 30.7, 31.0, 33.6, 44.6 (CH_2); 46.2, 47.1 (CH); 66.3 (CH_2); 79.4 (C); 128.2, 128.6 (CH); 136.0, 156.0, 172.3, 173.5 (C). FAB-MS: 898 (14, $[2M + H]^+$), 449 (100, $[M + H]^+$), 349 (59). Anal. calc. for $C_{25}H_{40}N_2O_5$ (448.60): C 66.94, H 8.99, N 6.24; found: C 66.97, H 9.00, N 6.19.

Boc-(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-OBn (**10**). Dipeptide **9** (2.24 g, 5.0 mmol) was dissolved in CH_2Cl_2/CF_3COOH 1:1 (10 ml) and stirred at r.t. for 2 h. The solvent was evaporated, the residue dissolved in CH_2Cl_2 and washed with sat. K_2CO_3 soln. (2 ×). The org. phase was evaporated, and the resulting free amine dipeptide was treated with **5b** (1.29 g, 5.3 mmol), HOBt (815 mg, 6.0 mmol), NMM (0.70 ml, 6.0 mmol), and EDC (960 mg, 5.0 mmol) in THF (25 ml) according to *GP 5* to yield, after FC (AcOEt/hexane 2:1, then AcOEt), **10** (2.67 g, 93%). White solid. M.p. 136–139°. $[\alpha]_D^{r.t.} = +6.3$ (c = 1.09, $CHCl_3$). IR ($CHCl_3$): 3500–3150 (br.), 3433*m*, 1729*m*, 1698*s*, 1656*s*, 1509*s*, 1454*m*, 1368*m*, 1168*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–0.90 (*m*, 2 Me_2CH); 1.13 (*d*, J = 6.6, Me); 1.19–1.28 (*m*, Me_2CHCH_2); 1.33–1.49 (*m*, Me_2CHCH_2); 1.44 (*s*, Me_3C); 1.55–1.73 (*m*, 2 CH_2); 1.84–1.97 (*m*, CH_2, CH); 2.06–2.28 (*m*, 2 $C(O)CH_2$); 2.38–2.51 (*m*, $C(O)CH_2$); 3.37–3.44 (*m*, CHN); 3.95–4.08 (*m*, 2 CHN); 4.41 (*d*, J = 10.1, CO_2NH); 5.11 (*s*, CH_2O); 6.04 (*d*, J = 8.4, NH); 6.54 (*d*, J = 9.0, NH); 7.28–7.37 (*m*, arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.7, 19.2, 21.3, 22.3, 23.1 (Me); 25.0 (CH); 28.4 (Me); 28.8, 30.8, 31.0 (CH_2); 32.7 (CH); 33.4, 33.4, 44.6 (CH_2); 44.8, 46.9, 54.9 (CH); 66.2 (CH_2); 79.4 (C); 128.1, 128.2, 128.5, (CH); 136.1, 156.7, 172.4, 172.5, 173.5 (C). FAB-MS: 1152 (7, $[2M + H]^+$), 576 (100, $[M + H]^+$), 476 (58). Anal. calc. for $C_{32}H_{53}N_3O_6$ (575.79): C 66.75, H 9.28, N 7.30; found: C 66.87, H 9.02, N 7.29.

Boc-(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-OH (**11**). The fully protected tripetide **10** (1.44 g, 2.5 mmol) was dissolved in MeOH (50 ml). Pd/C (10%) (150 mg) was added, the apparatus was evacuated and flushed three times with H_2 and the mixture stirred at r.t. for 24 h under H_2. Filtration through *Celite* and concentration under reduced pressure yielded crude **11** (1.19 g, 98%) as white solid, which was used without further purification. M.p. 146–147°. $[\alpha]_D^{r.t.} = +20.1$ (c = 0.73, $CHCl_3$). IR ($CHCl_3$): 3500–3200 (br.), 3429*m*, 2965*m*, 1697*s*, 1656*s*, 1506*s*, 1454*m*, 1368*m*, 1168*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.87–0.91 (*m*, 2 Me_2CH); 1.12 (*d*, J = 6.6, Me); 1.21–1.28 (*m*, Me_2CHCH_2); 1.37–1.51 (*m*, Me_2CHCH_2); 1.45 (*s*, Me_3C); 1.55–1.72 (*m*, 2 CH_2); 1.77–1.94 (*m*, CH_2, CH); 2.15–2.40 (*m*, 3 $C(O)CH_2$); 3.25–3.32 (*m*, CHN); 3.85–4.00 (*m*, 2 CHN); 6.37 (*d*, J = 9.7, CO_2NH); 7.72 (*d*, J = 9.1, NH); 7.77 (*d*, J = 8.3, NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.5, 19.7, 21.2, 22.4, 23.7 (Me); 26.1 (CH); 28.8 (CH_2); 28.9 (Me); 31.9, 32.2, 33.6, 33.7, 34.0 (CH_2); 34.2 (CH); 45.5 (CH_2); 45.8, 48.2, 56.5 (CH); 79.8, 158.7, 175.1, 175.3, 177.2 (C). FAB-MS: 994 (51, $[2M + Na]^+$), 972 (30, $[2M + H]^+$), 508 (100, $[M + Na]^+$), 486 (98), $[M + H]^+$, 386 (66).

Boc-(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-OBn (**12**). The fully protected tripeptide **10** (115 mg, 0.20 mmol) was dissolved in sat. HCl/dioxane (1 ml) and stirred at r.t. for 1 h. The solvent was evaporated, the residue dried under h.v. and treated with **11** (97 mg, 0.20 mmol), HOBt (36 mg, 0.26 mmol), NMM (0.07 ml, 0.60 mmol), and EDC (40 mg, 0.21 mmol) in THF (5 ml) according to *GP 5* to yield, after FC (CH_2Cl_2/MeOH 20:1), **12** (137 mg, 73%). Colorless glass. M.p. 218–219°. $[\alpha]_D^{r.t.} = +33.1$ (c = 0.98, $CHCl_3$). IR ($CHCl_3$): 3429*m*, 3323*s*, 1728*m*, 1697*s*, 1648*s*, 1550*s*, 1513*s*, 1453*m*, 1368*m*, 1169*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.84–0.92 (*m*, 4 Me_2CH); 1.08 (*d*, J = 6.5, Me); 1.13 (*d*, J = 6.7, Me); 1.21–1.42 (*m*, 9 CH); 1.44 (*s*, Me_3C); 1.50–1.72 (*m*, 6 CH); 1.83–1.96 (*m*, 2 CH); 2.05–2.52 (*m*, 15 CH); 3.46–3.52 (*m*, CHN); 3.75–3.80 (*m*, CHN); 3.84–3.89 (*m*, CHN); 3.97–4.08 (*m*, 3 CHN); 4.44 (*d*, J = 10.6, NH); 5.09 (*s*, CH_2O); 5.48 (*d*, J = 9.3, NH); 7.00 (*d*, J = 9.4, NH); 7.12 (*d*, J = 9.9, NH); 7.25 (*d*, J = 8.8, NH); 7.27–7.36 (*m*, arom. H); 7.49 (*d*, J = 9.0, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.6, 17.8, 19.4, 19.5, 21.0, 22.1, 22.3, 22.7, 23.2, 23.4 (Me); 25.0 (CH); 27.4, 28.1 (CH_2); 28.4 (Me); 29.7, 30.8, 31.0, 31.4, 31.6, 31.7, 31.7, 32.7 (CH_2); 33.0, 33.1 (CH); 34.0, 34.6 (CH_2); 43.7, 44.4 (CH); 44.6 (CH_2); 45.7 (CH); 46.2 (CH_2); 46.5, 52.8, 54.0 (CH); 66.0 (CH_2); 79.5 (C); 128.0, 128.1, 128.5 (CH); 136.2, 156.7, 171.7, 171.9, 172.5, 172.7, 173.5, 173.6 (C). FAB-MS: 944 (100, $[M + H]^+$), 844 (80).

(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-(R)-γ-HHVal-(S)-γ-HHAla-(R)-γ-HHLeu-OH (**1**). The fully protected hexapeptide **12** (115 mg, 0.12 mmol) was dissolved in THF/AcOH (5 ml/1 ml), then Pd/C (10%) (20 mg) was added. The apparatus was evacuated and flushed three times with H_2 and the mixture stirred at r.t. for 20 h under H_2. Filtration through *Celite* and concentration under reduced pressure yielded the crude carboxylic acid which was dissolved in CH_2Cl_2/CF_3COOH 1:1 (2 ml) and stirred for 2 h at r.t. The solvent was evaporated and the resulting colorless oil purified by RP-HPLC (MeCN/H_2O (0.1% CF_3COOH); 0–2 min 30% MeCN, 2–5 min 30 to 40% MeCN, 5–12 min 40 to 99% MeCN) to give as a colorless glass free **1** as TFA salt (104 mg, 98%). $[\alpha]_D^{r.t.} = 0.0$ ($c = 0.39$, MeOH). ^{1}H-NMR (500 MHz, CD_3OD): 0.88–0.93 (*m*, 6 Me); 1.02 (*d*, $J = 6.8$, Me); 1.03 (*d*, $J = 6.8$, Me); 1.13 (*d*, $J = 6.7$, Me); 1.14 (*d*, $J = 6.6$, Me); 1.23–1.29 (*m*, 2 Me_2CHCH_2); 1.35–1.51 (*m*, 2 Me_2CHCH_2); 1.55–1.73 (*m*, 6 CH); 1.76–1.85 (*m*, 4 CH); 1.88–1.99 (*m*, 4 CH); 2.20–2.34 (*m*, 5 $C(O)CH_2$); 2.36–2.45 (*m*, $C(O)CH_2$); 3.03–3.07 (*m*, CHN); 3.63–3.66 (*m*, CHN); 3.86–4.01 (*m*, 4 CHN). ^{13}C-NMR (125 MHz, CD_3OD): 17.9, 18.5, 18.7, 19.7, 20.9, 21.0, 22.5, 22.7, 23.7, 23.8 (Me); 26.2, 26.3 (CH); 27.0, 28.9 (CH_2); 31.5 (CH); 31.8, 32.1, 32.7, 33.1, 33.4, 33.6, 33.8, 33.9 (CH_2); 34.0 (CH); 34.2, 34.2, 45.5, 46.0 (CH_2); 46.0, 46.1, 47.7, 48.2, 55.0, 58.5 (CH); 174.2, 174.8, 175.1, 175.2, 175.5, 177.2 (C). FAB-MS: 792 (73, $[M + K]^+$), 776 (34, $[M + Na]^+$), 754 (100, $[M + H]^+$).

7. *NMR Spectroscopy of γ-Hexapeptide* **1**. Sample: 12 mg of **1** dissolved in 0.6 ml of C_5D_5N. *1D-NMR* (*AMX500*): ^{1}H-NMR (500 MHz): 90-K data points, 128 scans, 5.6-s acquisition time. {^{1}H}-BB-decoupled ^{13}C-NMR (125 MHz): 80-K data points, 795 transients, 1.3-s acquisition time, 45° excitation pulse, 1-s relaxation delay. Processed with 1.0-Hz exponential line broadening. *2D-NMR*. DQF.COSY (500 MHz, C_5D_5N) with pulsed field gradients (PFG) for coherence pathway selection [29]: *Acquisition:* 2K (t_2) × 512 (t_1) data points. 2 scans per t_1 increment, 0.14-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Processing:* Zero filling and FT to 2K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_2. TOCSY (DIPSI-2 SL; 10 kHz) [30] (500 MHz, C_5D_5N): *Acquisition:* 2K (t_2) × 512 (t_1) data points. 32 scans per t_1 increment, mixing time 125 ms, TPPI quadrature detection. *Processing:* Zero filling and FT to 2K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG [31] (500, 125 MHz, (D_5)pyridine): *Acquisition:* 2K (t_2) × 512 (t_1) data points, 4 scans per t_1 increment. ^{13}C-GARP Decoupling during t_2. 0.14-s acq. time in t_2. 1.5-s relaxation delay. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HMBC with PFG [32] (500, 125 MHz, (D_5)pyridine): *Acquisition:* no ^{13}C decoupling, otherwise identical to parameters for HSQC. *Processing:* Zero filling and FT to 1K × 1K after multiplication with cos^2 filter in ω_2 and gaussian filter in ω_1; power spectrum in both dimensions. ROESY [33] (500 MHz, (D_5)pyridine): *Acquisition:* A series of 3 ROESY spectra with mixing times of 50, 100, and 150 ms was acquired. CW-spin lock (3.8 kHz) between trim pulses, 4K (t_2) × 512 (t_1) data points, 48 scans per t_1- increment. 0.422-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication by sin^2 filter by $\pi/2$ in ω_2 and cos^2 filter in ω_1. Baseline correction with 3rd-degree polynomial in both dimensions.

8. *NMR Spectroscopy of γ-Hexapeptide* **1**. Sample: 12 mg of **1** dissolved in 0.6 ml of CD_3OH. *1D-NMR* (*AMX500*): ^{1}H-NMR (500 MHz): suppresssion of the CD_3OH signal by presaturation; 90-K data points, 128 scans, 5.6-s acquisition time. {^{1}H}-BB-decoupled ^{13}C-NMR (125 MHz): 80-K data points, 8000 transients, 1.3-s acquisition time, 45° excitation pulse, 1-s relaxation delay. Processed with 1.0-Hz exponential line broadening. *2D-NMR*. DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection [29] and solvent suppression: *Acquisition:* 2K (t_2) × 512 (t_1) data points. 2 scans per t_1 increment, 0.20-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. TOCSY (DIPSI-2 SL; 10 kHz) [30] (500 MHz, CD_3OH): *Acquisition:* 2K (t_2) × 512 (t_1) data points. 32 scans per t_1 increment, mixing time 125 ms, TPPI quadrature detection. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG [31] (500, 125 MHz, CD_3OH): *Acquisition:* 2K (t_2) × 512 (t_1) data points, 4 scans per t_1 increment. ^{13}C-GARP Decoupling during t_2. 0.20-s acquisition time in t_2. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and sin filter shifted by $\pi/2$ in ω_1. HMBC with PFG [32] (500, 125 MHz, CD_3OH): *Acquisition:* solvent suppression by presaturation, no ^{13}C decoupling, otherwise identical to parameters for HSQC. *Processing:* Zero filling and FT to 1K × 1K after multiplication with cos^2 filter in ω_2 and gaussian filter in ω_1: power spectrum in both dimensions. ROESY [33] (500 MHz, CD_3OH): *Acquisition:* A series of 3 ROESY spectra with mixing times of 50, 100, and 150 ms was acquired. Solvent suppression by presaturation, CW-spin lock (3.8 kHz) between trim pulses, 4K (t_2) × 768 (t_1) data points, 32 scans per t_1 increment. 0.405-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to

4K × 2K real/real data points after multiplication by $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\cos^2$ filter in ω_1. Baseline correction with 3rd-degree polynomial in both dimensions.

9. *NMR Structure Determination.* Calculations were performed using *X-PLOR 3.851* [34] on a *Silicon Graphics* O_2 (R 10000) workstation under Irix 6.3. Visualization and manipulation were carried out using Visual Molecular Dynamics (VMD) [35] and MolMol [17].

10. *Structure of γ-Hexapeptide* **1** *in* C_5D_5N. The parameter and topology files for γ-amino acids were generated from scratch, the parameter were taken according to parallhdg.pro [34]. A starting structure was generated by X-PLOR (*generate.inp*). 42 NOEs (*cf. Table 2*) were ordered according to their cross-peak volume in the contour plot of the 150-ms ROESY in three categories: strong, medium, and weak with 3.0 Å, 3.5 Å, and 4.5 Å, resp., as upper bound distance restraints and their *van der Waals* radii as lower bound distance restraints. The diastereotopic $CH_2(\beta)$ protons of all residues were stereospecifically assigned such that the smaller ^3J values result from coupling between H–C(γ) and H_{Re}–C(β). The diastereotopic $CH_2(\alpha)$ protons of residues 1, 2, 3, and 4 were stereospecifically assigned. The *ab initio* protocol *sa.inp* [36] of X-PLOR 3.851 was used to generate 20 structures. Initial time: 1000 K, 6000 high steps, 3000 cooling steps, 3-fs time step, NOE scaling 70, all other parameters were left unchanged. The resulting structures converged well to a right-handed helical structure. 3 H-bond restraints were added to the 42 NOE restraints during the refinement using the protocol *refine.inp* of X-PLOR 3.851. The resulting structures were analyzed using the protocol *accept.inp* with the following acceptance criteria: no NOE violations > 0.3 Å, rms difference for bond deviations from ideality < 0.01 Å, rms difference for angle deviations from ideality < 2°. All structures fulfilled this test. Mean global RMSD for all heavy backbone atoms of residues 2 to 5: 0.30 ± 0.14 Å, for all heavy atoms of residues 2 to 5: 0.77 ± 0.25 Å. We chose this bundle of structures as representative for the structure in solution.

REFERENCES

[1] D. Seebach, J. L. Matthews, *J. Chem. Soc., Chem. Commun.*, **1997**, *21*, 2015.

[2] G. Quinkert, E. Egert, C. Griesinger, 'Aspects of Organic Chemistry-Structure', VHCA, Basel, and VCH, Weinheim, 1996, p. 198–232.

[3] D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1998**, *81*, 932.

[4] G. Helmchen, in 'Houben-Weyl: Stereoselective Synthesis', Eds. G. Helmchen, R. W. Hoffmann, J. Mulzer, and E. Schaumann, Georg Thieme Verlag, Stuttgart-New York, 1996, Chapt. 1.

[5] P. Buchschacher, J.-M. Cassal, A. Fürst, W. Meier, *Helv. Chim. Acta* **1977**, *60*, 2747.

[6] C. C. Tseng, S. Terashima, S. Yamada, *Chem. Pharm. Bull.* **1977**, *25*, 29.

[7] S. Hanessian, R. Schaum, *Tetrahedron Lett.* **1997**, *38*, 163.

[8] J. Jurczak, A. Golebiowski, *Chem. Rev.* **1989**, *89*, 149.

[9] M. Smrcina, P. Majer, E. Majerova, T. A. Guerassina, M. A. Eisenstat, *Tetrahedron* **1997**, *53*, 12867.

[10] J. Podlech, D. Seebach, *Liebigs Ann.* **1995**, 1217, and lit. cit. therein.

[11] J. L. Matthews, C. Braun, C. Guibourdenche, M. Overhand, D. Seebach, in E. Juaristi, 'Enantioselective Synthesis of β-Amino Acids', Wiley-VCH, New York, 1997, pp. 105–126.

[12] J. Podlech, D. Seebach, *Angew. Chem.* **1995**, *107*, 507; *ibid., Int. Ed. Engl.* **1995**, *34*, 471.

[13] J.-A. Fehrentz, B. Castro, *Synthesis* **1983**, 676.

[14] W. D. Lubell, H. Rapoport, *J. Am. Chem. Soc.* **1987**, *109*, 236.

[15] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.

[16] D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 2043.

[17] R. Koradi, M. Billeter, K. Wüthrich *J. Mol. Graphics* **1996**, *14*, 51.

[18] D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi Jr, S. H. Gellman, *Nature (London)* **1997**, *387*, 381.

[19] D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071.

[20] M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy, S. L. Schreiber, *J. Am. Chem. Soc.* **1992**, *114*, 6568.

[21] C. E. Wintner, *J. Chem. Educ.* **1983**, *60*, 550.

[22] W. Klyne, V. Prelog, *Experientia* **1960**, *16*, 521.

[23] P. Koenig, T. J. Richmond, *J. Mol. Biol.* **1993**, *233*, 139.

[24] H. N. Rydon, *J. Chem. Soc.* **1964**, 1328; M. Kajtár, V. Bruckner, *Tetrahedron Lett.* **1966**, 4813; M. Kajtár, M. Hollósi, *Acta Chim. Acad. Sci. Hung.* **1970**, *65*, 403; T. Watanabe, T. Ina, K. Ogawa, T. Matsumoto, S. Sawa, S. Ono, *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3939.

[25] P. K. Sengupta, J. H. Bieri, M. Viscontini, *Helv. Chim. Acta* **1975**, *58*, 1374; C. N. C. Drey, G. P. Priestley, *J. Chem. Res. Miniprint* **1979**, 3055; V. L. Styles, J. L. Kelley, *J. Heterocycl. Chem.* **1990**, *27*, 1809; G. M. F. Bisset, K. Pawelczak, A. L. Jackman, A. H. Calvert, L. R. Hughes, *J. Med. Chem.* **1992**, *35*, 859.

[26] M. Kajtár, M. Hollósi, G. Snatzke, *Tetrehedron* **1971**, *27*, 5659; M. Hollósi, M. Kajtár, *Acta Chim. Acad. Sci. Hung.* **1972**, *73*, 247.

[27] A. Rosowsky, R. A. Forsch, J. H. Freisheim, P. V. Danenberg, R. G. Moran, M. M. Wick, *J. Med. Chem.* **1986**, *29*, 1872.

[28] P. Lombardi, *Chem. Ind. (London)* **1990**, Nov. 5, 708; S. Moss, *ibid.* **1994**, Feb. 21, 122.

[29] A. L. Davis, E. D. Laue, J. Keeler, D. Moskau, J. Lohman, *J. Magn. Reson.* **1991**, *94*, 637.

[30] S. P. Rucker, A. J. Shaka, *Mol. Phys.* **1989**, *68*, 509.

[31] A. L. Davis, J. Keeler, E. D. Laue, D. Moskau, *J. Magn. Reson.* **1992**, *98*, 207.

[32] W. Willker, D. Leibfritz, U. Kerssebaum, W. Bermel, *J. Magn. Reson.* **1993**, *31*, 287.

[33] C. Griesinger, R. R. Ernst, *J. Magn. Reson.* **1987**, *75*, 261.

[34] A. T. Brünger, in 'X-PLOR Manual V3.0', Eds. Y. University, New Haven, 1992.

[35] W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graphics* **1996**, *14*, 33.

[36] M. Nilges, G. M. Clore, A. M. Gronenborn, *FEBS Lett.* **1988**, *239*, 129; M. Nilges, J. Kuszewski, A. T. Brünger, in 'Computational Aspects of the Study of Macromolecules by NMR', Eds. J. C. Hoch, F. M. Poulsen, and C. Redfield, Plenum Press, New York, 1991.

Received April 1, 1998