Synthesis, CD Spectra, and Enzymatic Stability of β^2-Oligoazapeptides Prepared from (*S*)-2-Hydrazino Carboxylic Acids Carrying the Side Chains of Val, Ala, and Leu

by **Gérald Lelais**[1]) and **Dieter Seebach***

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH Hönggerberg, HCI, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich
(phone: +41 1 632 29 90; fax: +41 1 632 11 44; e-mail: seebach@org.chem.ethz.ch)

Dedicato al Professore *Duilio Arigoni* in occasione del suo 75° compleanno

β-Peptides offer the unique possibility to incorporate additional heteroatoms into the peptidic backbone (*Figs. 1* and *2*). We report here the synthesis and spectroscopic investigations of β^2-peptide analogs consisting of (*S*)-3-aza-β-amino acids carrying the side chains of Val, Ala, and Leu. The hydrazino carboxylic acids were prepared by a known method: Boc amidation of the corresponding *N*-benzyl-L-α-amino acids with an oxaziridine (*Scheme 1*). Couplings and fragment coupling of the 3-benzylaza-β^2-amino acids and a corresponding tripeptide (*N*-Boc/*C*-OMe strategy) with common peptide-coupling reagents in solution led to β^2-di, β^2-tri-, and β^2-hexaazapeptide derivatives, which could be *N*-debenzylated (**4**–**9**; *Schemes 2–4*). The new compounds were identified by optical rotation, and IR, ^{1}H- and ^{13}C-NMR, and CD spectroscopy (*Figs. 4* and *5*) and high-resolution mass spectrometry, and, in one case, by X-ray crystallography (*Fig. 3*). In spite of extensive measurements under various conditions (temperatures, solvents), it was not possible to determine the secondary structure of the β^2-azapeptides by NMR spectroscopy (overlapping and broad signals, fast exchange between the two types of NH protons!). The CD spectra of the *N*-Boc and *C*-OMe terminally protected hexapeptide analog **9** in MeOH and in H_2O (at different pH) might arise from a (*P*)-3_{14}-helical structure. The *N*-Boc-β^2-tri and *N*-Boc-β^2-hexaazapeptide esters, **7** and **9**, were shown to be stable for 48 h against the following peptidases: pronase, proteinase K, chymotrypsin, trypsin, carboxypeptidase A, and 20S proteasome.

1. Introduction. – The synthesis of non-natural peptide mimics, so called peptidomimetics, has received increasing attention in recent years. Their rapid development is driven by the fact that they will eventually replace both α-peptide substrates of enzymes and α-peptide ligands of protein receptors, due to the enhanced resistance toward proteases, increased bioavailability, and reduced immunogenicity they may have, as compared to α-peptide analogs. In addition, many of these oligomers adopt defined secondary structures, a necessary feature for promising candidates in drug design.

Among all synthetic strategies, the modification of the peptide backbone seems to be the most relevant for the construction of secondary structures [1]; this may involve isosteric or isoelectronic exchange of units (*e.g.*, peptoids, azapeptides, azatides, ethane-1,2-amines, oxazolidin-2-ones, and pyrrolidinones) or the introduction of additional fragments (*e.g.*, β-peptides, α-aminoxy peptides, hydrazino peptides, γ-peptides,

[1]) Part of the projected Ph.D. thesis of *G. L.*, ETH-Zürich 2004.

oligocarbamates, oligoureas, vinylogous sulfonopeptides, and many more other compounds) (*Fig. 1*)[2]).

β-Peptides are probably the most thoroughly investigated peptidomimetic oligomers. They are stable to rat metabolism [2], exhibit slow microbial degradation [3], and are inherently stable to a range of proteases and peptidases [4–8]. Additionally, as few as six amino acids are necessary to allow the peptides to fold into well-ordered secondary structures consisting of helices, turns, and sheets [9–13]. To expand the prolific field of β-peptides towards the construction of possible new secondary structures, the replacement of the C^{α} and C^{β} atoms of the β-amino acids with heteroatoms could be an attractive modification. In fact, oligomers of α-aminoxy acids, *i.e.*, β-amino acids where the C^{β}-atom is replaced by O, display a defined helical structure in solution, characterized by 1.8 residues per turn and H-bonds between the CO of residue i and NH of residue $i+2$. The so-formed eight-membered H-bonded rings (consecutive N–O turns) differ from the helices found in 'normal' β-peptides [14–17]. Another feasible modification would be the replacement of the C^{β}-atom of β-peptides by NR, leading to β^2-azapeptides, also called hydrazino peptides. These novel peptides are of great interest, owing to the possibility for additional H-bonds *via* the sp^3-N-atom. Quantum-chemical calculations carried out on 3-aza-β-homoglycine oligomers by *Günther* and *Hofmann* [18] show the presence of a wide variety of secondary-structure elements. Whereas some characteristics correspond to those structural types found in β-peptides, others are new. Initial experimental studies on this class of peptides were attempted by *Cheguillaume et al.* [19] in synthesizing N^{α}-substituted hydrazino acetic acid oligomers (R = Me, ^{i}Pr, ^{i}Bu). These achiral analogs of β^3-peptides were studied by NMR techniques and expected to fold to a helical structure containing eight-membered H-bonded rings, where both N-atoms are part of the ring[3]). This feature has effectively been proved previously by *Aubry* and co-workers [20–27] while studying structural and conformational aspects of α-hydrazino acids and mixed dipeptide derivatives by combined use of ^{1}H-NMR and IR spectroscopy, X-ray diffraction, and molecular dynamics. The following features should be noted: *i*) When both N-atoms are acylated, the two amide groups are in a rigid orthogonal disposition in order to minimize steric hindrance. *ii*) When only the N^{β}-atom is acylated, the N^{α}-atom has sp^3 character (bond angles are clearly smaller than 120°), and adopts either the (*R*)- or (*S*)-configuration according to the possible interactions in which the $N-H^{\alpha}$ bond could be involved. In fact, such proton-accepting and -donating properties result in a variety of inter- and intramolecular contacts. *iii*) The conformational arrangement of the $N^{\alpha}-N^{\beta}$ bond in the hydrazide group is much more flexible than in the diacylated compound. Instead of the single orthogonal arrangement, the $CO-N^{\beta}-N^{\alpha}-C^{\alpha}$ torsional angle effectively assumes values between 80° and 130° (absolute values), with a preference for 120°. *iv*) The hydrazide group can participate in a complex H-bond network in which the $CO-NH^{\beta}$ amide moiety resembles a standard peptide group, with a proton-donating site – the $N-H^{\beta}$ bond – and a proton-accepting site – the

---

[2]) A detailed discussion with extensive references will be given in a forthcoming review article by *D. Seebach*, *A. K. Beck*, and *D. Bierbaum* in *Angew. Chem.*

[3]) It should be noted that the presence of pyramidal N-atoms, *i.e.*, stereogenic centers with 'free' configuration, renders any folded structure chiral.

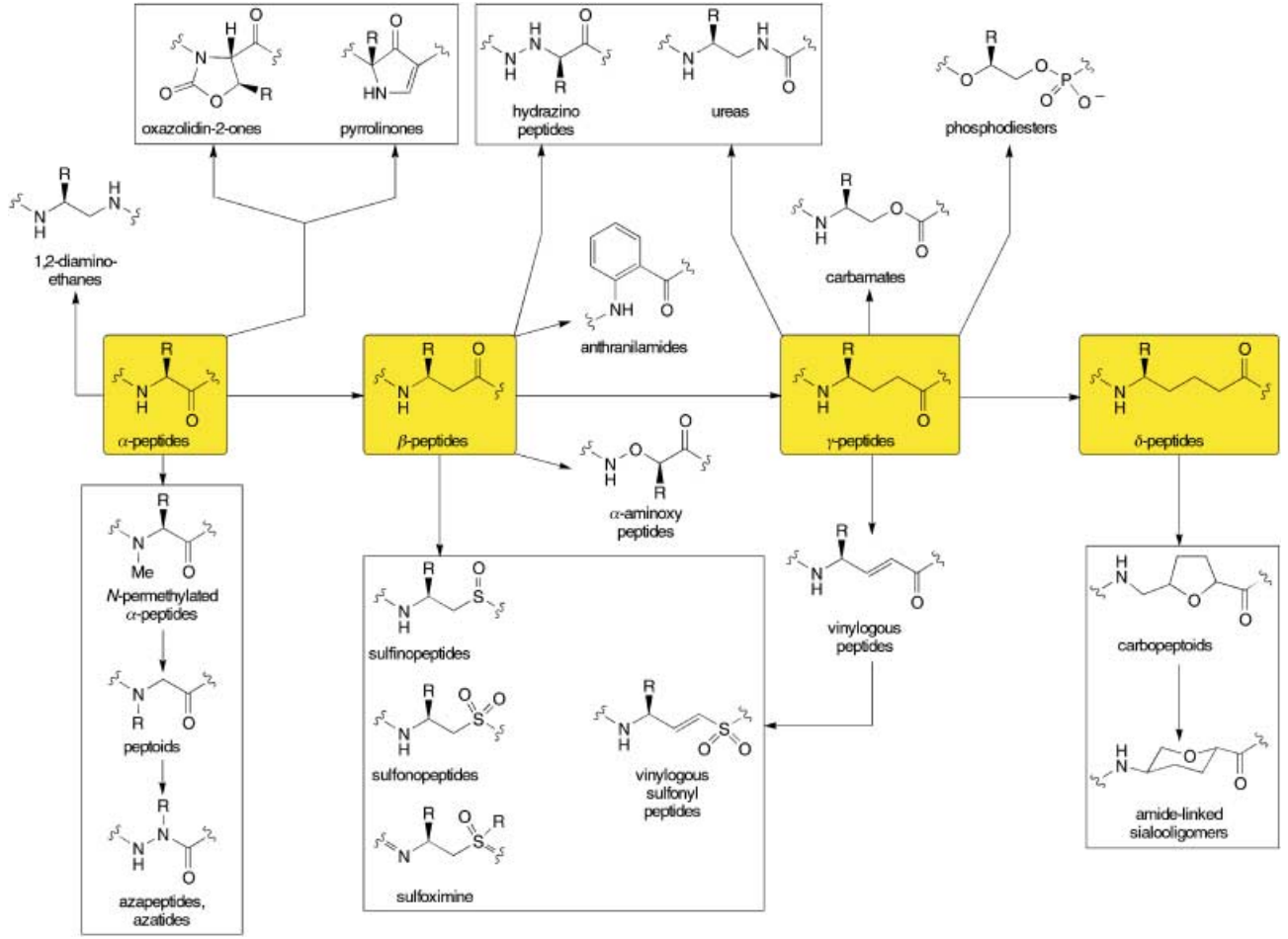


Fig. 1. *Structural lineage of peptidomimetics backbones.* The family tree of peptidomimetic backbones is organized horizontally from left to right by the number of atoms separating peptide (or peptide-like) units and vertically by the functional group classes. For clarity, each peptidomimetic is represented by the residue formula of only one configuration.

carbonyl O-atom. The sp^3-N^{α}-atom is both a proton-accepting site, and a proton donor when protonated; the lone pair is able to participate in the stabilization of a particular turn[4]) containing a double intramolecular interaction and eight atoms in the H-bonded ring.

Due to the fact that these peptides have not received much attention from the scientific community, and that the folding propensity of this type of peptides still needs to be investigated experimentally, we present in this paper the synthesis of chiral hydrazino oligomers with Val, Ala, and Leu side chains by coupling the 3-aza-β^2-amino acid derivatives in solution, and their comparison with analogous β-peptides (*Fig. 2*). In addition, spectroscopic and enzymatic-cleavage investigations are described.

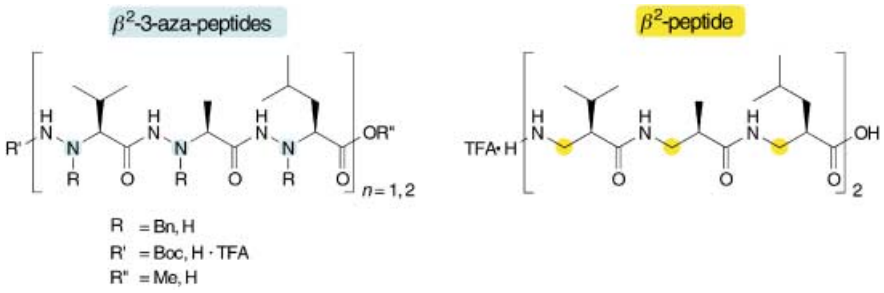


Fig. 2. *Structural comparison of β^2-aza- and β^2-peptides*

2. Preparation of 3-Aza-β^2-Amino Acids and Peptides. – Several methods for the synthesis of 3-aza-β^2-homoamino acids are described in the literature. Typically, they can be synthesized by reduction of *N*-nitrosoamino acids [29–32], asymmetric synthesis [33–39], *N*-amidation [26][40–43], the *Shestakov* urea rearrangement [44–46], or *Mitsunobu* reaction [47][48]. Thus, most hydrazino analogs of the coded α-amino acids have been prepared before.

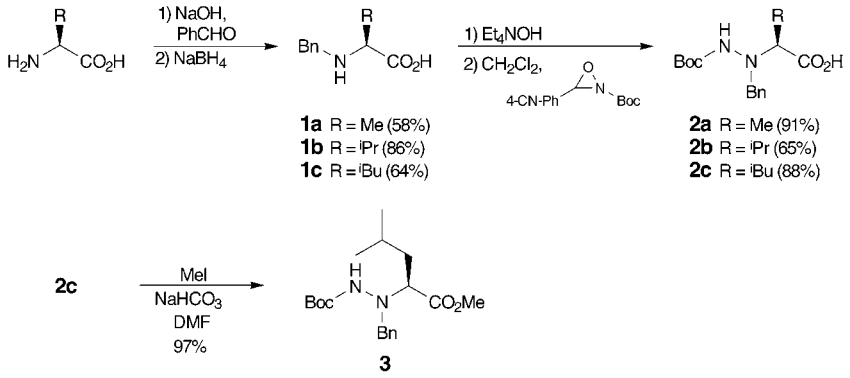
To attempt the synthesis of the β-amino acid derivatives needed for the hydrazino peptide coupling in solution, we decided to use an established method patented by *Collet et al.* [43]. This approach involves the *N*-amidation of secondary amines (in our case the *N*-benzyl-L-α-amino acids **1a**–**1c**) with a Boc-protected oxaziridine as electrophile [49]. The use of enantiomerically pure α-amino acids as starting materials for the preparation of the Boc-protected 3-benzylaza-β^2-homoamino acids is very convenient due to their ready availability. Compounds **1a**–**1c** were prepared in acceptable-to-good yields by formation of the imine from the corresponding α-amino acids (H-Ala-OH, H-Val-OH, and H-Leu-OH) and benzaldehyde, and subsequent $NaBH_4$ reduction [50][51] (*Scheme 1*). Isolation problems were encountered only with the alanine derivative **1a**, due to its solubility in H_2O at any pH. Therefore, **1a** could be

---

[4]) With reference to the so called γ-turn in α-peptides, in which an intramolecular $i+1 \rightarrow i-1$ H-bond closes a seven-membered ring [28], the α-hydrazino acid residue in position i exhibits a similar folded conformation, characterized by an intramolecular bifurcated H-bond in which the $N-H_{i+1}$ bond interacts both with the CO_{i-1} (to form an eight-membered cycle) and with the lone pair of the N_i^{α}-atom. This has been referred to as an expanded γ-like turn. The involvement of the $N-H_{i+1}$ proton in a double interaction is possibly responsible for the stability of this folded structure.

isolated in acceptable yield only after purification by ion-exchange chromatography. Further reaction of these intermediates with *N*-[(*tert*-butoxy)carbonyl]-3-(4-cyanophenyl)oxaziridine in CH_2Cl_2 at 0° [43] afforded **2a**–**2c** in good yields. Ester protection of the leucine derivative **2c** (for the C-terminal residue), with MeI in the presence of $NaHCO_3$ in DMF at room temperature, afforded **3** in nearly quantitative yield after purification (*Scheme 1*).

Scheme 1. *Preparation of the Boc-Protected 3-Benzylaza-β²-homoamino Acids* **2a**–**2c** *and of the Ester* **3** *from Natural* L-*α-Amino Acids*



Slow cooling and evaporation of a CH_2Cl_2/$^iPr_2O$ mixture from a solution of the *α*-hydrazino acid **2a** led to the formation of crystals suitable for structure determination by single-crystal X-ray diffraction (*Fig. 3*). The crystal structure[5]) was determined with SHELXS-97 [52] and displays some interesting features: the pyramidal sp^3 character of the N^{α}-atom is clearly demonstrated by the magnitude of its three bond angles, the sum of which is *ca.* 335°. This N-atom is able to participate in the stabilization of the eight-membered H-bonded ring formed by the carbazate CO and the COOH H-atom (bifurcated H-bond) and, therefore, assumes a (*S*)-configuration (*Fig. 3*,*a*). On the other hand, the NH^{β} H-atom is involved in an intermolecular H-bond with a COOH CO group of another molecule. This conformation probably experiences additional stabilization by an intramolecular CH-*π* contact[6]) between the Bn ring and the MeCH of the Boc group. The crystal packing displays an additional intermolecular CH-*π* contact between the Me group of one molecule and the Bn ring of another (*Fig. 3*,*b* and *c*).

With all the required aza-amino acids in hand, we envisaged the synthesis of various oligomers under the same coupling conditions as for *β*-peptides. Consequently, the ester **3** was Boc-deprotected by treatment with CF_3COOH (TFA) in CH_2Cl_2 and then submitted to 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC)/1-hydroxy-1*H*-benzotriazole (HOBt) coupling conditions with the Boc-protected amino acid **2a** (*Scheme 2*). The resulting dipeptide **4** could be isolated in

---

5) We thank Dr. *B. Schweizer* for the determination of the X-ray crystal structure.

6) For reviews on CH-*π* interactions, see [53][54].

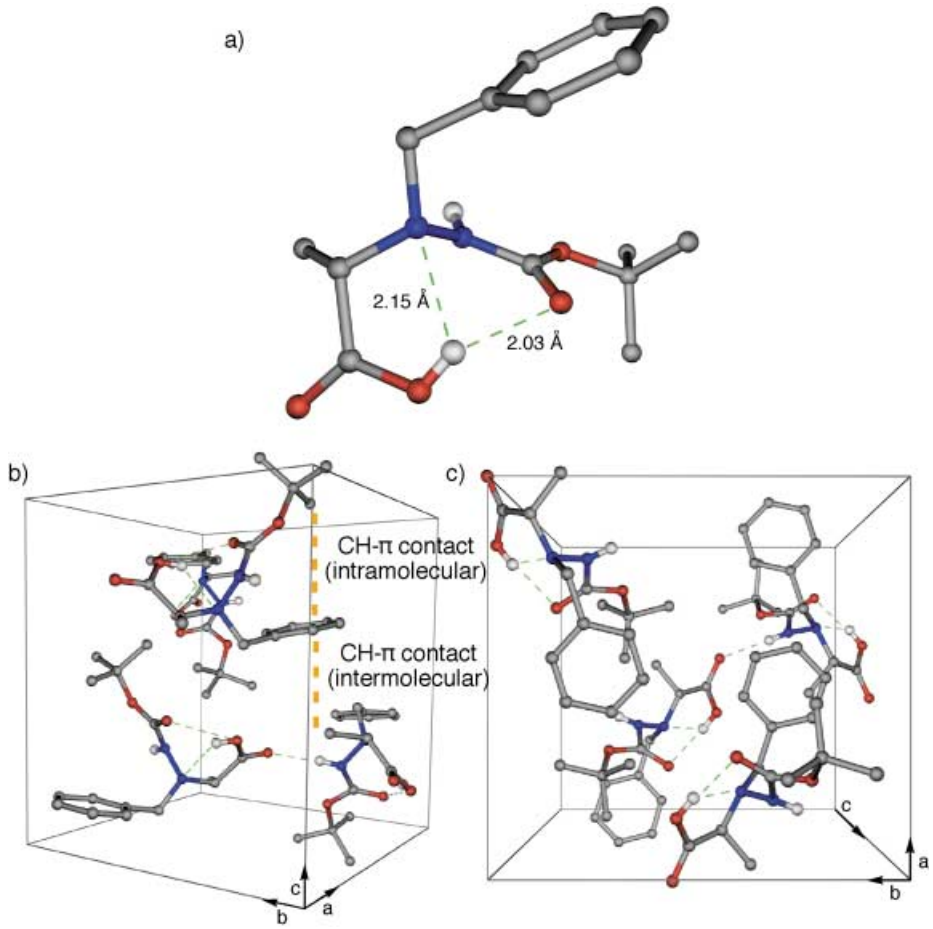
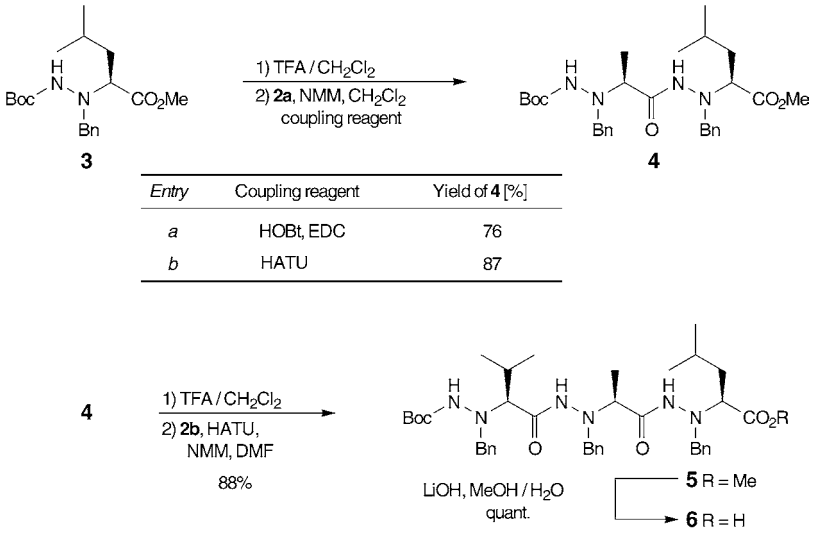


Fig. 3. *X-Ray crystal structure of* **2a**. *a*) The N^{α}-atom has a pyramidal conformation with (*S*)-configuration and participates in the stabilization of the eight-membered H-bonded ring formed by the carbazate CO and the COOH H-atom (bifurcated H-bond). *b*) Side-view (along the *a*,*b*-plane), and *c*) top-view (along the *c* axis) of the unit cell. The molecules are interconnected by H-bonds between the NH^{β} H-atom of one molecule and a COOH O-atom of another. The packing is additionally stabilized by an intramolecular CH-π contact between the Bn ring and the MeCH of the Boc group, and an intermolecular CH-π contact between the Me group and the Bn ring of two different molecules.

good yield, even though the reaction was not clean. Following Boc deprotection of **4** and reaction with **2b**, coupling with EDC and HOBt gave **5** in disappointing yield, the main product isolated being the valine active ester derivative as well as the unreacted Boc-deprotected dipeptide (data not shown).

Better results were obtained with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as coupling reagent. It is known that its presence accelerates the coupling reaction of amino acids, probably through intramolecular base catalysis [55][56]. Indeed, the reaction of the Boc-deprotected

Scheme 2. *Preparation of the β^2-Azapeptide Derivatives* **4**–**6**



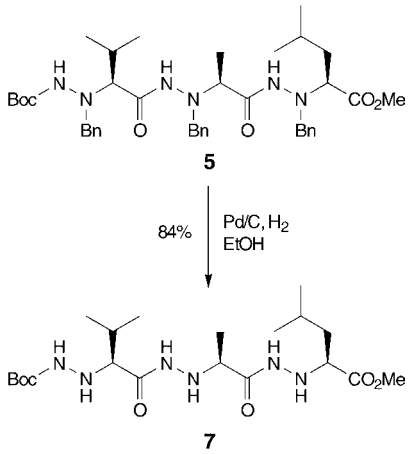
Entry	Coupling reagent	Yield of **4** [%]
a	HOBt, EDC	76
b	HATU	87

dipeptide with **2b**, HATU, and *N*-methylmorpholine (NMM) in DMF gave, after purification, the tripeptide **5** in 88% yield (*Scheme 2*). As the use of HATU avoided competitive side reactions, the same coupling conditions were also used for the synthesis of the dipeptide **4**. Boc Deprotection of **3**, followed by the reaction with **2a**, HATU, and NMM in CH_2Cl_2, led to the isolation of dipeptide **4** in 87% yield (*Scheme 2*). For fragment coupling, the methyl ester group of **5** was saponified by treatment with LiOH in MeOH/H_2O [57]. The resulting Boc-protected tripeptide derivative **6** was obtained in quantitative yield and with no loss of stereochemical integrity. Catalytic hydrogenolysis of the *N*-Bn groups of **5** with H_2 in EtOH in the presence of 10% Pd/C at room temperature gave the debenzylated Boc-tripeptide ester **7** in 84% yield (*Scheme 3*), without any N–N bond cleavage.

Unfortunately, attempts at subsequent cleavage of the Boc and methyl ester groups to prepare the fully deprotected tripeptide were in vain. Neither saponification of the ester group in **7**, followed by Boc deprotection, nor Boc deprotection of the acid **6**, followed by catalytic hydrogenation, led to the desired product.

The last step required for the synthesis of the fully protected hexapeptide **8** was the fragment coupling, which involved the reaction of the peptide acid **6** with the TFA salt derived from **5**. The use of HATU and NMM for this coupling reaction resulted in an inseparable mixture of products, none of which seemed to be the desired compound. Further attempts with other reagents were, therefore, investigated: the use of EDC/HOBt resulted in the isolation of the active ester of the acid **6** as major product; the reaction with EDC and DMAP (4-(dimethylamino)pyridine) resulted, again, in an inseparable mixture of products, and the use of PyBroP (bromotris(pyrrolidine)phosphonium hexafluorophosphate) afforded the peptide **8** in only 19% yield. Step-by-step coupling was also undertaken and shown to be inefficient upon scale-up, due to the formation of inseparable by-products at each step. The problem could be partially

Scheme 3. *Multiple* N-*Debenzylation of the Fully Protected Tripeptide Analog* **5**



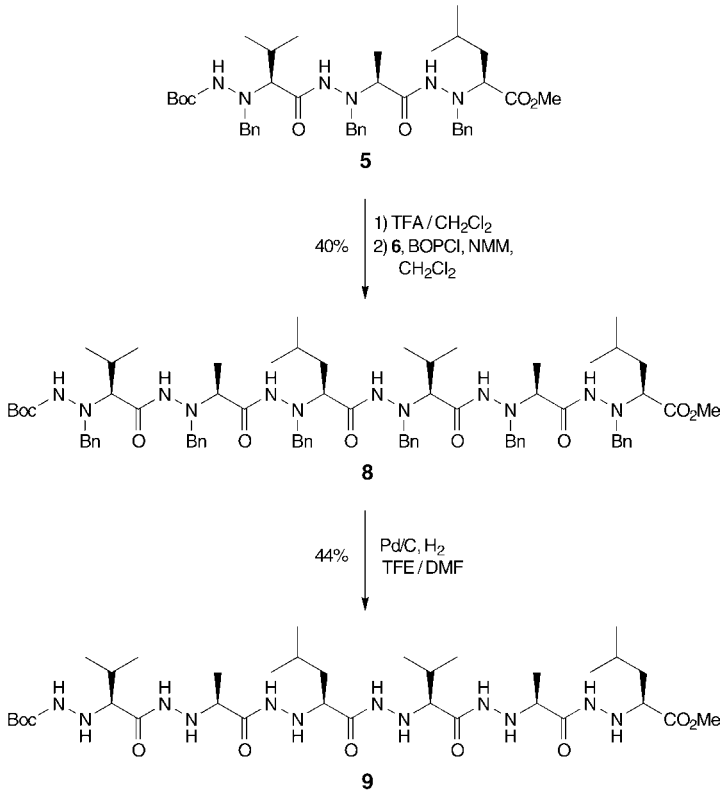
solved when BOPCl (bis(2-oxooxazolidin-3-yl)phosphinic chloride) and NMM were used for the coupling reaction. In fact, the peptide **8** could, thus, be isolated after column chromatography in reasonable yield and purity (*Scheme 4*). The hydrogenolysis of the N–Bn bonds in **8** with H_2 in CF_3CH_2OH/DMF 1:1, with *ca.* 30% Pd/C, afforded, after preparative HPLC and without any N–N bond cleavage, the peptide **9** in 44% yield. For the NMR measurements (*vide infra*), part of the compound had to be washed with aqueous bicarbonate solution to remove TFA.

3. Spectroscopic Investigations towards Secondary-Structure Elucidation. – Circular dichroism (CD) is extensively used to obtain information on the secondary structure of peptides and proteins in solution. For α-peptides and proteins, characteristic CD patterns are assigned to β-sheet, α-helix, and random-coil structures. When many secondary-structure motifs are present at the same time, only the population of each is extracted from the CD data. In the β-peptide world, the correlation is somehow more difficult. Even though certain patterns of the CD spectra have been correlated with secondary structures, such as 3_{14}-, 2.5_{12}-, and *12/10*-helices, or hairpin turns [58–60], there are limitations. It seems that only the 3_{14}-helix can be derived unambiguously from the CD spectra: it has been established, by numerous CD measurements and corresponding NMR investigations, that β-peptides consisting of L-β^3-amino acids exhibit a characteristic CD pattern with a negative *Cotton* effect near 215 nm, a zero-crossing between 205 and 210 nm, and a peak near 200 nm; this pattern is assigned to a left-handed (*M*)-3_{14}-helical structure [59][60]. The *12/10*-helix [12][61][62] and the hairpin turn [59] both show a maximum at around 200 nm and are, therefore, similar to that of a random-coil structure (in H_2O) [63].

β^2-Azapeptides are new in the field of peptidomimetics and should be compared with the β^2-peptide analogs only with due care[7]). As a first step in probing the existence

[7]) Experience in our group has led us to wait for NMR solution-structure determinations before we try to correlate structures with CD data [64].

Scheme 4. *Preparation of the β²-Hexaazapeptide Derivative* **8** *by Fragment Coupling of* **5** *with* **6** *and Subsequent Debenzylation to the Terminally Protected β²-Hexaazapeptide* **9**



of a secondary structure, CD spectra (0.2M solutions) of the fully protected (**5** and **8**) and of the *N*-debenzylated peptides (**7** and **9**) were recorded. As indicated by the CD spectra (normalized), none of the Bn-protected peptides show a significant *Cotton* effect. In fact, they show hardly any difference in comparison with the simple amino acid derivative **3**. In addition, going from **5** (three aza-amino acid residues) to **8** (six residues), the intensity of the CD curve actually decreases (*Fig. 4*, *left*)! On the other hand, the debenzylated peptides **7** and **9** (*Fig. 4*, *right*) give rise to a totally different effect: the CD spectra in MeOH of both peptides show, with different intensities, a maximum at *ca.* 225 nm, a zero-crossing at *ca.* 216 nm, and a more-intense minimum at *ca.* 198 nm.

Additional CD measurements with the peptide **9** in H_2O display a pattern similar to that obtained in MeOH and show a moderate pH dependence: in acidic media the curve is superimposable to that obtained in MeOH, and, at higher pH, it becomes somewhat less intense. It should be noted that the *β*-peptide **A**[8]), which was assigned [62][65] a (*P*)-3_{14}-helical structure, shows a similar CD pattern, with the peak being

[8]) The *β*-peptide **A** represents the terminally deprotected analog of **9**.

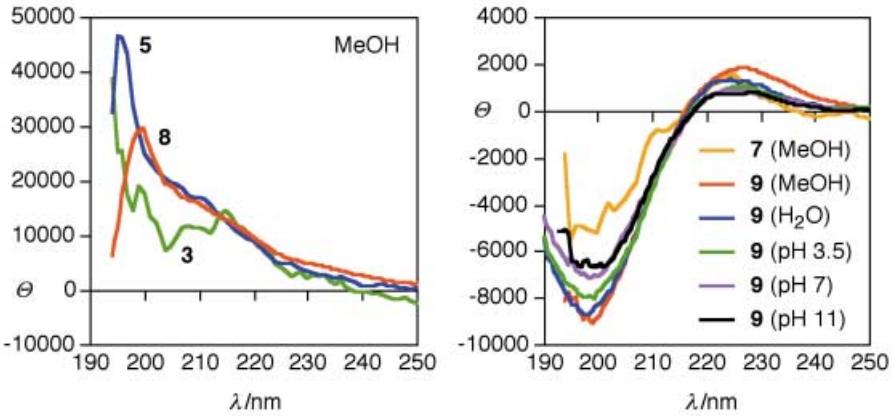


Fig. 4. *CD Spectra of the 3-benzylaza-β²-amino acid ester* **3** *and the azapeptide derivatives* **5**, *and* **7**–**9**. *Left:* The normalized CD spectra (0.2 mM in MeOH) of the fully protected amino acid **3**, and of the fully protected peptides **5** and **8** do not show any representative *Cotton* effects. *Right:* The CD curves (normalized) of the Bn-deprotected peptides **7** and **9** show, with different intensities, a peak at *ca.* 225 nm, a zero-crossing at *ca.* 216 nm, and a trough at *ca.* 198 nm. CD Curves of **9** are similar in MeOH and in H_2O; at acidic pH (blue and green) the curves are almost superimposable with the pattern in MeOH (red), a decrease in intensity is observed at basic pH. All CD measurements were performed at 22° on a *JASCO J-710* spectropolarimeter in a 1-mm cell, with 0.2 mM solutions.

blue-shifted by *ca.* 10 nm (*Fig. 5*; not normalized). Thus, from the CD spectra shown in *Fig. 4* we would have to conclude that our 'azapeptide' also forms a right-handed helical secondary structure.

To obtain more reliable[7]) information about the secondary structure of the new peptide analogs, NMR investigations of the hexapeptide **9** have also been undertaken,

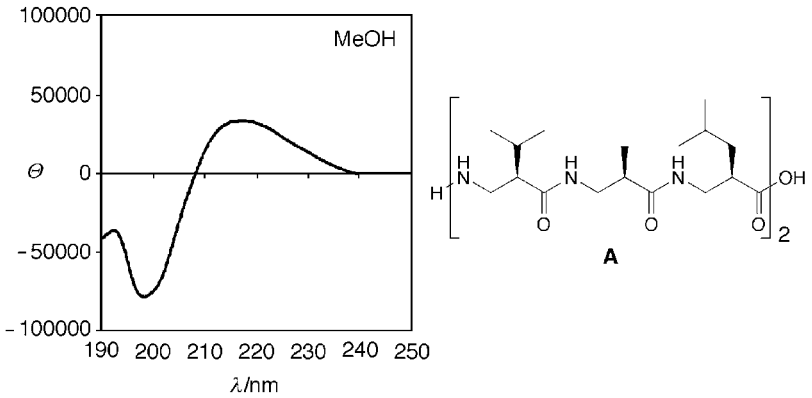


Fig. 5. *CD Spectrum and formula of the β²-hexapeptide* **A** *in MeOH*. The pattern consists of a positive *Cotton* effect at *ca.* 216 nm, a zero-crossing at *ca.* 210 nm, and a negative peak at *ca.* 198 nm, and it is believed to build to some extend a (*P*)-3_{14}-helix. The CD spectrum was measured at −20°, resulting in a 40% increase of the molar ellipticity of the 216-nm *Cotton* effect in comparison with the data collected at 22° [62]. Concentration 0.2 mM, molar ellipticity Θ in 10 deg cm² mol⁻¹.

unfortunately without success. A strong overlapping of the NH^{β} (peptidic) signals and the *absence of signals* from the NH^{α} (hydrazino) H-atoms characterized the ^{1}H-NMR spectrum of the peptide **9** (as TFA salt) in $CDCl_3$. Measurements in CD_2Cl_2 at various temperatures (between $-70°$ and room temperature) did not solve the problem: the signals of the peptidic H-atoms were overlapping, and the hydrazino NH^{α} signals were still absent; in addition, all other signals were degenerate at low temperatures. On the other hand, the ^{1}H-NMR spectrum of the TFA-free hexapeptide **9** in CD_2Cl_2 displayed much better dispersion of the peptidic H-atoms, but the signals from the NH^{α} H-atoms were broad and overlapping. Attempts to improve the resolution in other deuterated solvents were not successful. In all cases, the NH H-atoms appeared as broad *singlets*, probably due to fast exchange between both types of NH H-atoms, and structural assignment was impossible.

4. Enzymatic Stability of the Tri- and Hexapeptide Analogs 7 and 9. – Despite the fact that no secondary structure could be assigned by NMR spectroscopy, we consider these peptides to be interesting candidates as peptidomimetics. In the early seventies, two natural peptides containing a α-hydrazino acid residue were isolated: linatine from linseed [30][66] and negamycin [67][68], a mushroom antibiotic, containing the hydrazino analogues of proline and sarcosine. Moreover, α-hydrazino acids are also known to be potent inhibitors of pyridoxal phosphate-dependent enzymes (transaminases and decarboxylases) through the probable formation of a hydrazone with the pyridoxal phosphate aldehyde group [36][69–71]. For example, Carbidopa (α-hydrazino-α-(3,4-dihydroxybenzyl)propanoic acid) is used for the treatment of *Parkinson*'s disease as a dopa-decarboxylase inhibitor [72]. Since proteolytic stability is a prerequisite for possible pharmacological applications, we wondered whether these hydrazino peptides would be as stable as the β-peptides [4–8]. We have, therefore, tested compounds **7** and **9** *in vitro* with several peptidases: pronase, proteinase K, chymotrypsin, trypsin, carboxypeptidase A, and 20S proteasome (human, yeast, arch. flug.)[9]). We were pleased to observe that both compounds are stable for at least 48 h under conditions where α-peptides are degraded within minutes!

5. Conclusions. – We have shown that chiral α-hydrazino carboxylic acid derivatives can be coupled in solution for the preparation of β^2-azapeptides. The α-hydrazino derivative **2a** displays, in the solid state, the characteristic bifurcated H-bond, which might be responsible for the formation of helical conformations in corresponding peptides. The CD spectra of the fully protected tri- and hexapeptides, **5** and **8**, respectively, do not show any significant *Cotton* effect, indicating lack of folding into any secondary structure. On the other hand, the *N*-debenzylated peptides **7** and **9** display patterns similar to those of the corresponding β^2-hexapeptide **A**, albeit with lower intensities and a red-shifted maximum. Unfortunately, no secondary-structure determination was possible by NMR techniques: there is fast H-exchange between both types of NH H-atoms and insufficient dispersion of their signals. Interestingly, both hydrazino peptides **7** and **9** are stable against peptidases and make this class of peptides interesting candidates for drug design.

---

[9]) We gratefully acknowledge the help of Dr. *J. Frackenpohl* for conducting these experiments.

Experimental Part

1. *General.* Abbreviations: BOPCl: Bis(2-oxooxazolidin-3-yl)phosphinic chloride, DMAP: 4-(dimethylamino)pyridine, EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: (flash chromatography), HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01 – 0.1 Torr, IEC: ion-exchange chromatography (*Amberlite IR-120*), NMM: *N*-methylmorpholine. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*) and from KOH (Et_2O), resp. Et_3N was distilled from CaH_2 and stored over 4-Å molecular sieves. MeI was filtered over Alox before use. Amino acids were purchased from *Fluka* or *Senn*. All other reagents were used as received from *Fluka*. TLC: *Merck* silica gel *60* F_{254} plates; detection with UV, anisaldehyde soln. (9.2 ml of anisaldehyde, 12.5 ml of conc. H_2SO_4, 3.75 ml of AcOH, 340 ml of EtOH), or 'Mo-stain' soln. (25 g of phosphormolybdic acid, 10 g of $Ce(SO_4)_2 \cdot H_2O$, 60 ml of conc. H_2SO_4, 940 ml of H_2O), FC: *Fluka* silica gel *60* (40 – 63 μm); at *ca.* 0.2 bar. Anal. HPLC: *Knauer* HPLC system (pump type *WellChrom K-1000 Maxy-Star*, degasser, UV detector (variable-wavelength monitor) or *Merck HPLC system* (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*), *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250 × 4 mm) or *Macherey-Nagel* C_{18} column (*Nucleosil 100-5* C_{18} (250 × 4 mm). Prep. HPLC: *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*). *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250 × 21 mm); TFA for prep. HPLC was used as UV-grade quality (> 99% GC). Lyophilization: *Heterosicc* cooling condenser with h.v. pump. M.p.: *Büchi-510* apparatus; uncorrected. CD Spectra: *Jasco J-710* recording from 190 to 250 nm at r.t.; 1-mm rectangular cell; average of five scans, corrected for the baseline; peptide concentration 0.2 mM; molar ellipticity Θ in $deg \cdot cm^2 \cdot dmol^{-1}$ (λ in nm); smoothing by *Jasco* software. IR Spectra: *Perkin-Elmer 1600 FT-IR* spectrophotometer. NMR Spectra: *Bruker AMX II 500* (^1H: 500 MHz, ^{13}C: 125 MHz), *Bruker AMX-400* (^1H: 400 MHz, ^{13}C: 100 MHz), *Bruker AMX-300* (^1H: 300 MHz, ^{13}C: 75 MHz), *Varian Mercury XL 300* (^1H: 300 MHz, ^{13}C: 75 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (= 0 ppm); *J* values in Hz. MS: *IonSpec Ultima 4.7 T FT* Ion Cyclotron Resonance (ICR, HR-MALDI, in 2,5-dihydroxybenzoic acid 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. *Synthesis of* N-*Bn-Protected α-Amino Acids: General Procedure 1* (*GP 1*). Similar to the procedures reported in [50][51], the α-amino acid (1 equiv.) was dissolved in 2N NaOH (1 equiv.), PhCHO (1 equiv.) was added, and the mixture was stirred at r.t. for 15 min. After cooling to 0°, $NaBH_4$ (0.33 equiv.) was added in one portion (*Caution:* strong development of gas!). One h later, PhCHO and $NaBH_4$ were added again according to the same procedure. After stirring 1 h more, the mixture was washed with Et_2O (2 ×), the aq. layer was saturated with solid NaCl and acidified to pH 7 in an ice-bath with 2N HCl. The white solid formed was collected, and the crude product was purified by washing with cold H_2O or by IEC.

3. *Synthesis of Boc-(*S*)-β²-3-aza(Bn)-Homoamino Acids: General Procedure 2* (*GP 2*). Similar to the procedure reported in [43], 1.5M methanolic soln. of Et_4NOH (1 equiv.) was added to *N*-benzyl-α-amino acid (1 equiv.). The suspension was stirred until the soln. became clear. The MeOH was evaporated, and the yellow oily residue was dissolved in CH_2Cl_2 (0.33M). The soln. was cooled to 0°, and *N*-[(*tert*-butoxy)carbonyl]-3-(4-cyanophenyl)oxaziridine (1.05 equiv., synthesised according to [49]), dissolved in CH_2Cl_2 (0.5M), was added dropwise. The flask was hermetically sealed and placed in a refrigerator for 12 – 24 h. The solvent was then evaporated, and the residue was taken up in an aq. NaOH soln. (12 mg of NaOH/50 ml of H_2O for 1 mmol). After stirring for 30 min, the precipitated 4-formylbenzonitrile was separated by filtration. The aq. phase was slightly salinized, washed with Et_2O (7 ×), and then acidified to pH 3 with solid $KHSO_4$. The resulting milky soln. was extracted with Et_2O (3 ×), the combined org. layers dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude product was either recrystallized or used without further purification.

4. *Boc Deprotection: General Procedure 3* (*GP 3*). Similar to the procedure reported in [4], the Boc-protected amino acid/peptide was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. An equal volume of TFA was added, and the mixture was stirred at 0° for 4 h (TLC control). The solvent was evaporated; the residue was taken up in dried toluene or $CHCl_3$, and evaporated twice. The resulting trifluoroacetate salt was dried under h.v., identified by NMR, and used in a next step without further purification.

5. *Peptide Coupling: General Procedures 4* (*GP 4*). *a*) The appropriate trifluoroacetate salt was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. This soln. was treated successively with NMM (3 – 5.3 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in CH_2Cl_2 (0.25M), and HATU or BOPCl (1 equiv.). The mixture was allowed to warm to r.t. and then stirred until TLC indicated complete reaction. Subsequent dilution with AcOEt was

followed by washing with 5% aq. $KHSO_4$ soln. (1×), 5% aq. $NaHCO_3$ soln. (1×) and sat. aq. NaCl-soln. (1×). The org. layer was dried ($MgSO_4$) and concentrated under reduced pressure. FC yielded the pure peptide.

b) The appropriate trifluoroacetate salt was dissolved in DMF (0.5M) and cooled to 0°. This soln. was treated successively with NMM (4 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in DMF (0.25M), and HATU (1 equiv.). The mixture was allowed to warm to r.t. and then stirred until TLC indicated complete reaction. Sat. aq. $NaHCO_3$ soln. was added, and the mixture was stirred for another 10 min. The mixture was diluted with H_2O and extracted with Et_2O (3×). The combined org. layers were washed with sat. aq. NaCl soln. (1×), dried ($MgSO_4$), and concentrated under reduced pressure. FC afforded the pure peptide.

6. *Bn Deprotection of the Hydrazino Group: General Procedure 5* (*GP 5*). The fully protected peptide was dissolved in EtOH or CF_3CH_2OH/DMF 1:1 (0.07–0.2M). Some drops of AcOH and *ca.* 10–30% (*w*/*w*) Pd/C (10%) were added. The apparatus was evacuated and flushed with H_2 (3×), and the mixture was stirred under H_2 atmosphere (balloon) for 4–6 d. Subsequent filtration through *Celite* and concentration under reduced pressure yielded the Bn-free peptide, which was further purified by FC or HPLC.

*(*S*)-2-(Benzylamino)propanoic Acid* (*Bn-Ala-OH*; **1a**). Alanine (6.24 g, 70.0 mmol) was transformed according to *GP 1* and purified by IEC according to the following procedure: *Amberlite IR-120* (250 g) was mixed with H_2O and packed in a chromatography column. The column was first washed with 2N HCl (8 l) (flow rate: 100–1000 ml/h) and then with H_2O (3 l) until the pH was neutral. Thereafter, the product was put on the column as a soln. in H_2O (250 ml), and washing was continued with additional H_2O (8.5 l). Finally, washing with 1M NH_4OH (1 l) eluted the product, which was concentrated under reduced pressure and lyophilized to yield **1a** (7.34 g, 58%). White powder. M.p. 248–249° (dec.) ([73]: m.p. 251). $[\alpha]_D^{r.t.} = +2.5$ ($c = 1.02$, 6N HCl) ([73]: $[\alpha]_D^{r.t.} = +4.3$ ($c = 1$, 6N HCl)). ^{1}H-NMR (300 MHz, D_2O): 1.47 (*d*, $J = 7.1$, Me); 3.67 (*q*, $J = 7.1$, CHN); 4.17 (*d*, $J = 13.0$, 1 H, PhCH_2); 4.23 (*d*, $J = 13.0$, 1 H, PhCH_2); 7.46 (*s*, 5 arom. H). The NMR data of the corresponding HCl salt are reported in [74] and are in agreement with the data of **1a**.

*(*S*)-2-(Benzylamino)-3-methylbutanoic Acid* (*Bn-Val-OH*; **1b**). Transformation of valine (3.52 g, 30.0 mmol) according to *GP 1* and washing with a large amount of cold H_2O yielded **1b** (5.35 g, 86%). The product was used in a next step without further purification. White solid. M.p. 256–257° ([73]: m.p. 255°). $[\alpha]_D^{r.t.} = +10.5$ ($c = 1.02$, 6N HCl) ([73]: $[\alpha]_D^{r.t.} = +14.8$ ($c = 1$, 6N HCl)). ^{1}H-NMR (200 MHz, D_2O): 0.92 (*d*, $J =$ 7.1, Me); 0.98 (*d*, $J = 7.1$, Me); 2.00–2.20 (*m*, Me_2CH); 3.32 (*d*, $J = 4.6$, CHN); 4.07 (*d*, $J = 13.3$, 1 H, PhCH_2); 4.22 (*d*, $J = 13.3$, 1 H, PhCH_2); 7.46 (*s*, 5 arom. H). The NMR data of the corresponding HCl salt were reported in [74] and are in agreement with the data of **1b**.

*(*S*)-2-(Benzylamino)-4-methylpentanoic Acid* (*Bn-Leu-OH*; **1c**). Transformation of leucine (3.69 g, 30.0 mmol) according to *GP 1* and washing with a large amount of cold H_2O yielded **1c** (3.96 g, 64%). The product was used in a next step without further purification. White solid. M.p. 222–223° ([73]: m.p. 242). $[\alpha]_D^{r.t.} = +14.6$ ($c = 1.00$, 6N HCl) ([73]: $[\alpha]_D^{r.t.} = +14.2$ ($c = 1$, 6N HCl)). ^{1}H-NMR (300 MHz, D_2O): 0.84 (*d*, $J =$ 5.6, Me); 0.89 (*d*, $J = 5.6$, Me); 1.59–1.76 (*m*, Me_2CH); 3.56 (*m*, CHN); 4.15 (*d*, $J = 13.1$, 1 H, PhCH_2); 4.26 (*d*, $J = 13.1$, 1 H, PhCH_2); 7.48 (*s*, 5 arom. H). The physical data are in agreement with the values reported in [73].

*(*S*)-2-{1-Benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}propanoic Acid* (*(*S*)-Boc-β²-3-aza(Bn)-hAla-OH*; **2a**). Compound **1a** (500 mg, 2.80 mmol) was first treated with methanolic Et_4NOH (4.00 ml, 2.80 mmol) and then with *N*-[(*tert*-butoxy)carbonyl]-3-(4-cyanophenyl)oxaziridine (720 mg, 2.92 mmol) according to *GP 2*. Purification of the crude product by recrystallization (Et_2O/hexane) yielded **2a** (787 mg, 91%). White solid. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.34 (*s*, *t*-Bu); 1.41 (*d*, $J = 7.2$, Me); 3.63–3.73 (*m*, CHN); 3.99 (*s*, PhCH_2); 6.15 (br. *s*, 0.8 H, NH); 7.05 (br. *s*, 0.2 H, NH); 7.25–7.40 (*m*, 5 arom. H); 9.62 (br. *s*, OH). The physical data are in agreement with the values reported in [43].

*(*S*)-2-{1-Benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}-3-methylbutanoic Acid* (*(*S*)-Boc-β²-3-aza(Bn)-hVal-OH*; **2b**). Compound **1b** (2.07 g, 10.0 mmol) was first treated with methanolic Et_4NOH (6.67 ml, 10.0 mmol) and then with *N*-[(tert-butoxy)carbonyl]-3-(4-cyanophenyl)oxaziridine (2.59 g, 10.5 mmol) according to *GP 2*. Purification of the crude product by recrystallization (Et_2O/hexane) yielded **2b** (2.09 g, 65%). White crystals. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.98 (*d*, $J = 6.5$, Me); 1.10 (*d*, $J = 6.5$, Me); 1.38 (*s*, *t*-Bu); 2.05–2.17 (*m*, Me_2CH); 3.00–3.20 (br. *m*, CHN); 3.90–4.10 (br. *m*, PhCH_2); 6.55 (br. *s*, 0.5 H, NH); 7.13 (br. *s*, 0.5 H, NH); 7.24–7.30 (*m*, 3 arom. H); 7.31–7.42 (*m*, 2 arom. H); 8.06 (br. *s*, OH). The physical data are in agreement with the values reported in [43].

*(*S*)-2-{1-Benzyl-2-[(*tert*-butoxy)carbon yl]hydrazino}-4-methylpentanoic Acid* (*(*S*)-Boc-β²-3-aza(Bn)-hLeu-OH*; **2c**). Treatment of **1c** (2.14 g, 9.67 mmol) with methanolic Et_4NOH (6.5 ml, 9.75 mmol) and then with *N*-[(*tert*-butoxy)carbonyl]-3-(4-cyanophenyl)oxaziridine (2.50 g, 10.2 mmol) according to *GP 2* yielded **2c** (2.87 g, 88%). The crude product was used in a next step without further purification. White foam. ^{1}H-NMR

(300 MHz, $CDCl_3$): 0.77 (br. *s*, Me); 0.91 (*d*, *J* = 6.2, Me); 1.36 (*s*, *t*-Bu); 1.36–1.49 (*m*, 1 H, CH_2); 1.67–1.77 (*m*, 1 H, CH_2); 2.07 (br. *s*, Me_2CH); 3.46–3.54 (br. *m*, CHN); 3.92–4.18 (br. *m*, $PhCH_2$); 6.51 (br. *s*, NH); 7.24–7.39 (*m*, 5 arom. H); 9.56 (br. *s*, OH).

*Methyl (*S*)-2-{1-Benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}-4-methylpentanoate* (*(*S*)-Boc-β²-3-aza(Bn)-hLeu-OMe*; **3**). Similar to the procedure reported in [75], compound **2c** (2.66 g, 7.91 mmol) was dissolved in DMF (0.61M), and solid $NaHCO_3$ (1.33 g, 15.8 mmol) was added, followed by MeI (0.79 ml, 12.7 mmol). After stirring at r.t. for 25 h, H_2O was added, and the mixture was extracted with AcOEt (4×). The combined org. layers were successively washed with 5% aq. Na_2SO_3-soln. (1×) and sat. aq. NaCl-soln. (1×), and dried ($MgSO_4$). Removal of the solvent and FC (Et_2O/pentane 1:6) yielded **3** (2.69 g, 97%). Colorless oil. R_f (Et_2O/Pentan 1:6) 0.20. $[\alpha]_D^{r.t.} = +25.4$ (*c* = 0.99, $CHCl_3$). IR ($CHCl_3$): 3364*w*, 3008*w*, 2958*m*, 2868*w*, 1730*s*, 1696*s*, 1483*m*, 1455*m*, 1435*m*, 1392*m*, 1368*m*, 1159*s*, 1077*w*, 1050*w*, 1028*w*, 993*w*. ^{1}H-NMR (300 MHz, DMSO, 90°): 0.80 (*d*, *J* = 6.5, Me); 0.87 (*d*, *J* = 6.5, Me); 1.31 (*s*, *t*-Bu); 1.40 (*ddd*, *J* = 14.0, 8.1, 5.6, 1 H, Me_2CHCH_2); 1.62 (*ddd*, *J* = 14.0, 8.7, 5.3, 1 H, Me_2CHCH_2); 1.85–1.97 (*m*, Me_2CH); 3.52 (*dd*, *J* = 8.7, 5.6, CHN); 3.69 (*s*, MeO); 3.95 (*s*, $PhCH_2$); 7.05 (br. *s*, NH); 7.20–7.32 (*m*, 3 arom. H); 7.35–7.39 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.10, 23.29 (Me); 23.98 (CH); 28.20 (Me); 39.19 (CH_2); 51.48 (Me); 61.31 (CH_2); 62.25 (CH); 79.49 (C); 127.49, 128.18, 129.45 (CH); 136.99, 155.06, 174.81 (C). MALDI-MS: 373.2 (7.20), 317.1 (12.72), 295.0 (3.77), 251.2 (17.65), 191.1 (14.95), 91.0 (8.15). Anal. calc. for $C_{19}H_{30}N_2O_4$ (350.46): C 65.12, H 8.63, N 7.99; found: C 64.94, H 8.58, N 8.18.

*Methyl (*S*)-2-[1-Benzyl-2-((*S*)-2-{1-benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}propionyl)hydrazino]-4-methylpentanoate* (*Boc-(*S*)-β²-3-aza(Bn)-hAla-(*S*)-β²-3-aza(Bn)-hLeu-OMe*; **4**). Compound **3** (766 mg, 2.18 mmol) was Boc-deprotected according to *GP 3*. The resulting trifluoroacetate salt was coupled with **2a** (644 mg, 2.18 mmol), NMM (0.73 ml, 6.63 mmol), and HATU (831 mg, 2.18 mmol) according to *GP 4a* for 24 h. FC (CH_2Cl_2/Et_2O 10:1) yielded **4** (1.00 g, 87%). White foam. R_f (CH_2Cl_2/Et_2O 10:1) 0.20. $[\alpha]_D^{r.t.} = +30.8$ (*c* = 1.00, $CHCl_3$). IR ($CHCl_3$): 3340*w*, 3008*m*, 2960*m*, 1727*s*, 1687*m*, 1494*m*, 1368*m*, 1160*s*, 996*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.78 (*d*, *J* = 6.5, Me); 0.90 (*d*, *J* = 6.7, Me); 1.20 (*d*, *J* = 7.0, Me); 1.33 (*s*, *t*-Bu); 1.29–1.46 (*m*, 1 H, Me_2CHCH_2); 1.66–1.73 (*m*, 1 H, Me_2CHCH_2); 1.99–2.09 (br. *m*, Me_2CH); 3.32–3.35 (*m*, CHN); 3.54–3.58 (*m*, CHN); 3.78 (*s*, MeO); 3.81 (*s*, $PhCH_2$); 3.98 (*s*, $PhCH_2$); 6.29 (br. *s*, NH); 7.20–7.32 (*m*, 8 arom. H); 7.40 (*d*, *J* = 6.6, 2 arom. H); 8.36 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.87, 21.29, 23.20 (Me); 24.17 (CH); 28.24 (Me); 39.28 (CH_2); 51.65 (Me); 60.06 (CH_2); 61.16, 62.50 (CH); 79.71 (C); 127.51, 127.62, 128.24, 128.27, 129.24, 129.25 (CH); 136.59, 155.34, 171.83, 174.38 (C). MALDI-MS: 549.3 (3.41), 449.2 (100.00), 427.3 (38.93), 358.2 (9.04), 329.2 (16.17), 305.2 (59.85), 267.1 (24.87), 256.1 (41.70), 249.1 (10.47), 245.2 (21.36), 238.1 (15.30), 171.1 (12.31), 149.1 (23.27). Anal. calc. for $C_{29}H_{42}N_4O_5$ (526.67): C 66.14, H 8.04, N 10.64; found: C 66.25, H 8.18, N 10.67.

*Methyl (*S*)-2-(1-Benzyl-2-{(*S*)-2-[1-benzyl-2-((*S*)-2-{1-benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}-3-methylbutanoyl)hydrazino]propanoyl}hydrazino)-4-methylpentanoate* (*Boc-(*S*)-β²-3-aza(Bn)-hVal-(*S*)-β²-3-aza(Bn)-hAla-(*S*)-β²-3-aza(Bn)-hLeu-OMe*; **5**). Compound **4** (1.76 g, 3.34 mmol) was Boc-deprotected according to *GP 3*. The resulting trifluoroacetate salt was coupled with **2b** (1.08 g, 3.34 mmol) according to *GP 4b* for 46 h. FC (CH_2Cl_2/Et_2O 3:1) yielded **5** (2.15 g, 88%). White foam. CD (0.2 mM in MeOH) $+4.67 \cdot 10^4$ (195 nm). R_f (CH_2Cl_2/Et_2O 3:1) 0.21. $[\alpha]_D^{r.t.} = +50.3$ (*c* = 0.98, $CHCl_3$). IR ($CHCl_3$): 3335*w*, 3007*m*, 2962*m*, 1725*s*, 1679*s*, 1479*m*, 1368*m*, 1166*m*, 992*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.59 (*d*, *J* = 6.4, Me); 0.86 (*d*, *J* = 6.5, Me); 0.93 (*d*, *J* = 6.7, Me); 1.00 (*d*, *J* = 6.5, Me); 1.24 (*d*, *J* = 6.8, Me); 1.34 (*s*, *t*-Bu); 1.43–1.51 (*m*, 1 H, Me_2CHCH_2); 1.69–1.77 (*m*, 1 H, Me_2CHCH_2); 1.96–2.04 (br. *m*, Me_2CH); 2.04–2.29 (br. *m*, Me_2CH); 2.45–2.51 (br. *d*, CHN); 3.28–3.33 (br. *q*, CHN); 3.56–3.60 (br. *d*, CHN); 3.62 (*d*, *J* = 5.2, 1 H, $PhCH_2$); 3.64 (*d*, *J* = 5.2, 1 H, $PhCH_2$); 3.71 (*d*, *J* = 13.2, 1 H, $PhCH_2$); 3.79 (*s*, MeO); 3.89 (*d*, *J* = 13.2, 1 H, $PhCH_2$); 3.99 (*s*, $PhCH_2$); 6.99–7.21 (br. *s*, NH); 7.23–7.40 (*m*, 15 arom. H); 7.80–8.00 (br. *s*, NH); 8.00–8.20 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.90, 19.26, 20.23, 21.27, 23.22 (Me); 24.27 (CH); 28.26 (Me); 39.43 (CH_2); 51.79 (Me); 59.42 (CH); 59.74, 61.26 (CH_2); 62.90, 70.02 (CH); 79.28 (C); 127.37, 127.79, 127.82, 128.18, 128.32, 128.40, 128.50, 128.76, 128.91, 129.20, 129.30 (CH); 136.53, 137.15, 154.97, 171.30, 172.15, 174.57 (C). MALDI-MS: 753.4 (9.56), 653.4 (100.00), 631.4 (12.63), 519.3 (18.91), 471.3 (19.35), 434.2 (91.21), 410.2 (48.96), 375.2 (12.58), 342.2 (10.40), 305.2 (21.03), 258.1 (12.94), 245.2 (17.20), 217.8 (10.82), 199.1 (10.07), 177.1 (17.75), 175.1 (13.14), 166.1 (10.30). Anal. calc. for $C_{41}H_{58}N_6O_6$ (730.95): C 67.37, H 8.00, N 11.50; found: C 67.24, H 8.00, N 11.37.

*(*S*)-2-(1-Benzyl-2-{(*S*)-2-[1-benzyl-2-((*S*)-2-{1-benzyl-2-[(*tert*-butoxy)carbonyl]hydrazino}-3-methylbutyryl)hydrazino]propionyl}hydrazino)-4-methylpentanoic Acid* (*Boc-(*S*)-β²-3-aza(Bn)-hVal-(*S*)-β²-3-aza(Bn)-hAla-(*S*)-β²-3-aza(Bn)-hLeu-OH*; **6**). A soln. of the fully protected tripeptide **5** (250 mg, 0.34 mmol) in MeOH (0.2M) was cooled to 0°, treated with 2.7M LiOH soln. (0.76 ml, 2.05 mmol), and stirred at r.t. overnight. The mixture was diluted with H_2O, acidified to pH 2–3 with 1N HCl, and extracted with AcOEt (3×). The combined

org. layers were dried ($MgSO_4$) and concentrated under reduced pressure to yield **6** (245 mg, quant.). The acid was used in a next step without further purification.

*Methyl (*S*)-2-(2-{(*S*)-2-[2-((*S*)-2-{2-[(*tert*-Butoxy)carbonyl]hydrazino}-3-methyl-butanoyl)hydrazino]-propionyl}hydrazino)-4-methylpentanoate* (*Boc-(*S*)-β²-3-aza-hVal-(*S*)-β²-3-aza-hAla-(*S*)-β²-3-aza-hLeu-OMe*; **7**). Compound **5** (151 mg, 0.21 mmol) was debenzylated in EtOH (0.21M) with 10% Pd/C (10%) according to *GP 5* for 4 d. Purification by FC (CH_2Cl_2/MeOH 15:1) yielded **7** (79.4 mg, 84%). Colorless oil. CD (0.2 mM in MeOH): $-2.58\cdot10^3$ (198 nm); $+820$ (225 nm). R_f (CH_2Cl_2/MeOH 15:1) 0.27. IR ($CHCl_3$): 3407*m*, 2965*s*, 2873*m*, 1714*s*, 1673*s*, 1468*s*, 1392*w*, 1369*m*, 1158*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (*d*, *J* = 6.5, Me); 0.96 (*t*, *J* = 6.5, 2 Me); 1.03 (*d*, *J* = 7.1, Me); 1.33 (*d*, *J* = 6.8, Me); 1.42 (*s*, *t*-Bu); 1.50–1.65 (*m*, Me_2CHCH_2); 1.68–1.79 (*m*, Me_2CH); 2.00–2.10 (*m*, Me_2CH); 3.36 (*d*, *J* = 5.0, CHN); 3.55 (*q*, *J* = 6.8, CHN); 3.62 (*t*, *J* = 7.1, CHN); 3.73 (*s*, MeO); 4.00 (br. *s*, N*H*CH); 4.57 (br. *s*, N*H*CH); 4.96 (br. *s*, N*H*CH); 6.76 (*s*, NHCO); 8.43 (*s*, NHCO); 8.52 (*s*, NHCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 17.21, 18.44, 19.20, 22.39, 22.81 (Me); 24.96 (CH); 28.36 (Me); 30.40 (CH); 39.02 (CH_2); 52.42 (Me); 60.34, 61.28, 69.83 (CH); 80.80, 156.68, 171.53, 171.94, 173.55 (C). MALDI-MS: 411.3 (16.62), 383.2 (77.23), 337.2 (29.22), 280.2 (10.38), 254.1 (100.00), 195.1 (91.15), 168.1 (18.53), 163.1 (11.54), 149.1 (13.73). HR-MALDI-MS: 483.2907 ($[C_{20}H_{40}N_6NaO_6]^+$; calc. 483.2907).

*Boc-(*S*)-β²-3-aza(Bn)-hVal-(*S*)-β²-3-aza(Bn)-hAla-(*S*)-β²-3-aza(Bn)-hLeu-(*S*)-β²-3-aza(Bn)-hVal-(*S*)-β²-3-aza(Bn)-hAla-(*S*)-β²-3-aza(Bn)-hLeu-OMe* (**8**). Compound **5** (250 mg, 0.34 mmol) was Boc-deprotected according to *GP 3*. The resulting trifluoroacetate salt was coupled with **6** (245 mg, 0.34 mmol), NMM (0.20 ml, 1.82 mmol), and BOPCl (87.0 mg, 0.34 mmol) according to *GP 4a* for 29 h. Purification of the crude product by FC (CH_2Cl_2/Et_2O 3:1) yielded **8** (182 mg, 40%). White glass. CD (0.2 mM in MeOH): $+2.98\cdot10^4$ (199 nm). R_f (CH_2Cl_2/Et_2O 3:1) 0.26. IR ($CHCl_3$): 3326*m*, 3007*m*, 2961*m*, 1954*w*, 1877*w*, 1815*w*, 1724*s*, 1674*s*, 1477*s*, 1368*m*, 1160*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.54 (br. *d*, Me); 0.65 (*d*, *J* = 6.8, Me); 0.81 (br. *d*, Me); 0.86 (*d*, *J* = 6.5, Me); 0.89 (*d*, *J* = 6.5, Me); 0.94 (*d*, *J* = 6.5, Me); 0.97 (*d*, *J* = 6.5, Me); 1.11 (*d*, *J* = 6.5, Me); 1.22 (*d*, *J* = 7.5, 2 Me); 1.34 (*s*, *t*-Bu); 1.36–1.80 (*m*, 2 Me_2CHCH_2); 1.80–2.16 (br. *m*, 4 Me_2CH); 2.36–2.63 (*br. m*, 2 CHN); 3.17–3.32 (*m*, 2 CHN); 3.61–3.68 (*m*, 2 CHN); 3.72–3.92 (*m*, 4 $PhCH_2$); 3.78 (*s*, MeO); 3.83 (*s*, $PhCH_2$); 3.97 (*s*, $PhCH_2$); 6.99–7.01 (br. *d*, NH); 7.00–7.45 (*m*, 30 arom. H); 7.60–7.94 (br. *m*, 2 NH); 8.30–8.80 (br. *m*, 3 NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 16.98, 19.32, 20.41, 21.36, 21.93, 23.21, 23.36 (Me); 24.39 (CH); 28.36 (Me); 39.59 (CH_2); 51.91 (Me); 59.69 (CH); 60.10, 60.17, 60.31, 60.40, 60.76, 60.95, 61.52, 61.72 (CH_2); 62.90, 70.02 (CH); 79.24 (C); 127.23, 127.68, 128.06, 128.22, 128.37, 128.70, 128.92, 129.16, 129.32 (CH); 136.20, 137.09, 154.90, 155.64, 171.29, 171.79, 172.28, 174.45 (C). MALDI-MS: 1351.8 (10.66), 1251.7 (33.51), 1032.6 (19.05), 797.5 (40.52), 693.4 (12.53), 638.4 (27.96), 619.3 (12.47), 593.4 (100.00), 578.3 (42.01), 487.3 (11.66), 434.2 (40.31), 410.2 (17.16), 374.2 (10.44), 342.2 (15.79), 304.1 (11.04), 245.2 (17.23). HR-MALDI-MS: 1351.7937 ($[C_{76}H_{104}N_{12}NaO_9]^+$; calc. 1351.7947).

*Boc-(*S*)-β²-3-aza-hVal-(*S*)-β²-3-aza-hAla-(*S*)-β²-3-aza-hLeu-(*S*)-β²-3-aza-hVal-(*S*)-β²-3-aza-hAla-(*S*)-β²-3-aza-hLeu-OMe* (**9**). Compound **8** (76.2 mg, 0.06 mmol) was debenzylated in CF_3CH_2OH/DMF 1:1 (0.8 ml) with 30% Pd/C (10%) according to *GP 5* for 6 d. Purification by RP-HPLC (column: *Macherey-Nagel, Nucleosil 100-7 C_8*, 250 × 21 mm; linear gradient: MeCN/0.1% TFA in H_2O 30:70 → 60:40 in 30 min; detection: UV 215 nm; t_R 11.6 min) and lyophilization yielded **9** (19.8 mg, 44%). White powder. For anal. reasons, a small amount of the debenzylated peptide (10 mg) was dissolved in CH_2Cl_2, washed with semi-sat. aq. $NaHCO_3$ soln. (1×) and with sat. aq. NaCl soln. (1×), dried ($MgSO_4$), and concentrated under reduced pressure to afford the TFA-free **9** (7 mg). Colorless solid. CD (0.2 mM in MeOH): $-9.0\cdot10^3$ (198 nm); $+1.88\cdot10^3$ (226 nm). ^{1}H-NMR (500 MHz, C_5D_5N): 0.86 (*d*, *J* = 6.7, Me); 0.87 (*d*, *J* = 6.6, Me); 0.89 (*d*, *J* = 6.6, Me); 0.92 (*d*, *J* = 6.6, Me); 1.13 (*d*, *J* = 6.8, Me); 1.14 (*d*, *J* = 6.8, Me); 1.17 (*d*, *J* = 6.7, 2 Me); 1.23–1.31 (*m*, Me_2CHCH_2); 1.48 (*s*, *t*-Bu); 1.51 (*d*, *J* = 6.9, Me); 1.52 (*d*, *J* = 6.9, Me); 1.70–1.76 (*m*, Me_2CHCH_2); 1.87–1.95 (br. *m*, 2 Me_2CH); 2.28–2.53 (br. *m*, 2 Me_2CH); 3.72 (*s*, MeO); 3.77–3.80 (*m*, 2 CHN); 3.95–4.03 (*m*, 3 CHN); 4.14–4.15 (*m*, CHN); 5.31 (br. *s*, NH); 5.62 (br. *s*, NH); 5.73 (br. *s*, NH); 5.80 (br. *s*, NH); 9.06 (br. *s*, NH). ^{13}C-NMR (125 MHz, C_5D_5N): 17.47, 17.55, 18.92, 19.09, 19.52, 19.70, 22.41, 22.56, 22.81, 23.09 (Me); 25.20 (CH); 28.48 (Me); 30.72, 30.85 (CH); 40.26, 40.99 (CH_2); 51.84 (Me); 60.00, 62.35, 63.08, 70.37 (CH); 79.42, 157.89, 172.91, 173.14, 173.42, 173.52, 173.69, 174.38 (C). MALDI-MS: 811.5 (26.73), 737.4 (23.49), 711.5 (100.00), 529.4 (11.59), 475.3 (14.75), 329.2 (10.26). HR-MALDI-MS: 811.5096 ($[C_{34}H_{60}N_{12}NaO_9]^+$; calc. 811.5130).

REFERENCES

[1] D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893.
[2] H. Wiegand, B. Wirz, A. Schweitzer, G. P. Camenisch, M. I. Rodriguez-Perez, G. Gross, R. Woessner, R. Voges, P. I. Arvidsson, J. Frackenpohl, D. Seebach, *Biopharm. Drug Dispos.* **2002**, *23*, 251.
[3] J. V. Schreiber, J. Frackenpohl, F. Moser, T. Fleischmann, H. P. E. Kohler, D. Seebach, *ChemBioChem.* **2002**, *3*, 424.
[4] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.
[5] T. Hintermann, D. Seebach, *Chimia* **1997**, *51*, 244.
[6] D. Seebach, S. Abele, J. V. Schreiber, B. Martinoni, A. K. Nussbaum, H. Schild, H. Schulz, H. Hennecke, R. Woessner, F. Bitsch, *Chimia* **1998**, *52*, 734.
[7] J. Frackenpohl, P. I. Arvidsson, J. V. Schreiber, D. Seebach, *ChemBioChem* **2001**, *2*, 445.
[8] E. A. Porter, B. Weisblum, S. H. Gellman, *J. Am. Chem. Soc.* **2002**, *124*, 7324.
[9] D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015.
[10] K. Gademann, T. Hintermann, J. V. Schreiber, *Curr. Med. Chem.* **1999**, *6*, 905.
[11] R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219.
[12] M. Rueping, J. V. Schreiber, G. Lelais, B. Jaun, D. Seebach, *Helv. Chim. Acta* **2002**, *85*, 2577.
[13] K. Gademann, A. Häne, M. Rueping, B. Jaun, D. Seebach, *Angew. Chem., Int. Ed.* **2003**, *42*, 1534.
[14] D. Yang, F. F. Ng, Z. J. Li, Y. D. Wu, K. W. K. Chan, D. P. Wang, *J. Am. Chem. Soc.* **1996**, *118*, 9794.
[15] D. Yang, J. Qu, B. Li, F. F. Ng, X. C. Wang, K. K. Cheung, D. P. Wang, Y. D. Wu, *J. Am. Chem. Soc.* **1999**, *121*, 589.
[16] Y. D. Wu, D. P. Wang, K. W. K. Chan, D. Yang, *J. Am. Chem. Soc.* **1999**, *121*, 11189.
[17] C. Peter, X. Daura, W. F. van Gunsteren, *J. Am. Chem. Soc.* **2000**, *122*, 7461.
[18] R. Günther, H. J. Hofmann, *J. Am. Chem. Soc.* **2001**, *123*, 247.
[19] A. Cheguillaume, A. Salaun, S. Sinbandhit, M. Potel, P. Gall, M. Baudy-Floc'h, P. Le Grel, *J. Org. Chem.* **2001**, *66*, 4923.
[20] J. Viret, A. Collet, V. Pichonpesme, A. Aubry, *New J. Chem.* **1988**, *12*, 253.
[21] A. Aubry, M. Marraud, *Biopolymers* **1989**, *28*, 109.
[22] A. Lecoq, M. Marraud, A. Aubry, *Tetrahedron Lett.* **1991**, *32*, 2765.
[23] A. Aubry, D. Bayeul, J. P. Mangeot, J. Vidal, S. Sterin, A. Collet, A. Lecoq, M. Marraud, *Biopolymers* **1991**, *31*, 793.
[24] A. Aubry, J. P. Mangeot, J. Vidal, A. Collet, S. Zerkout, A. Lecoq, M. Marraud, *J. Cell. Biochem.* **1993**, 229.
[25] M. Marraud, V. Dupont, V. Grand, S. Zerkout, A. Lecoq, G. Boussard, J. Vidal, A. Collet, A. Aubry, *Biopolymers* **1993**, *33*, 1135.
[26] A. Aubry, J. P. Mangeot, J. Vidal, A. Collet, S. Zerkout, M. Marraud, *Int. J. Pept. Protein Res.* **1994**, *43*, 305.
[27] S. Zerkout, V. Dupont, A. Aubry, J. Vidal, A. Collet, A. Vicherat, M. Marraud, *Int. J. Pept. Protein Res.* **1994**, *44*, 378.
[28] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985**, *37*, 1.
[29] G. Pollak, H. Yellin, A. Carmi, *J. Med. Chem.* **1964**, *7*, 220.
[30] H. J. Klosterman, G. L. Lamoureux, J. L. Parsons, *Biochemistry* **1967**, *6*, 170.
[31] W. Lijinsky, L. Keefer, J. Loo, *Tetrahedron* **1970**, *26*, 5137.
[32] K. Achiwa, S. Yamada, *Tetrahedron Lett.* **1975**, *16*, 2701.
[33] C. Gennari, L. Colombo, G. Bertolini, *J. Am. Chem. Soc.* **1986**, *108*, 6394.
[34] D. A. Evans, T. C. Britton, R. L. Dorow, J. F. Dellaria, *J. Am. Chem. Soc.* **1986**, *108*, 6395.
[35] L. A. Trimble, J. C. Vederas, *J. Am. Chem. Soc.* **1986**, *108*, 6397.
[36] J. G. Kelland, L. D. Arnold, M. M. Palcic, M. A. Pickard, J. C. Vederas, *J. Biol. Chem.* **1986**, *261*, 13216.
[37] W. Oppolzer, R. Moretti, *Tetrahedron* **1988**, *44*, 5541.
[38] K. J. Hale, J. Cai, V. Delisser, S. Manaviazar, S. A. Peak, G. S. Bhatia, T. C. Collins, N. Jogiya, *Tetrahedron* **1996**, *52*, 1047.
[39] N. Xi, L. B. Alemany, M. A. Ciufolini, *J. Am. Chem. Soc.* **1998**, *120*, 80.
[40] J. Vidal, J. Drouin, A. Collet, *J. Chem. Soc., Chem. Commun.* **1991**, 435.
[41] J. Vidal, L. Guy, S. Stérin, A. Collet, *J. Org. Chem.* **1993**, *58*, 4791.
[42] D. A. Niederer, J. T. Kapron, J. C. Vederas, *Tetrahedron Lett.* **1993**, *34*, 6859.
[43] A. Collet, J. Vidal, J. C. Hannachi, L. Guy (CNRS France), Patent WO 9709303, 1997 (*Chem. Abstr.* **1997**, *126*, 264365).

[44] J. Viret, J. Gabard, A. Collet, *Tetrahedron* **1987**, *43*, 891.
[45] A. Amour, A. Collet, C. Dubar, M. Reboud-Ravaux, *Int. J. Pept. Protein Res.* **1994**, *43*, 297.
[46] L. De Luca, M. Falorni, G. Giacomelli, A. Porcheddu, *Tetrahedron Lett.* **1999**, *40*, 8701.
[47] J. A. Killian, M. D. Van Cleve, Y. F. Shayo, S. M. Hecht, *J. Am. Chem. Soc.* **1998**, *120*, 3032.
[48] N. Brosse, M. F. Pinto, J. Bodiguel, B. Jamart-Grégoire, *J. Org. Chem.* **2001**, *66*, 2869.
[49] J. Vidal, S. Damestoy, L. Guy, J. C. Hannachi, A. Aubry, A. Collet, *Chem.–Eur. J.* **1997**, *3*, 1691.
[50] P. Quitt, J. Hellerbach, K. Vogler, *Helv. Chim. Acta* **1963**, *46*, 327.
[51] H. G. Bossler, D. Seebach, *Helv. Chim. Acta* **1994**, *77*, 1124.
[52] G. M. Sheldrick, SHELXS-97, Georg-August-Universität Göttingen, Germany, 1997.
[53] M. Nishio, M. Hirota, *Tetrahedron* **1989**, *45*, 7201.
[54] M. Nishio, Y. Umezawa, M. Hirota, Y. Takeuchi, *Tetrahedron* **1995**, *51*, 8665.
[55] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397.
[56] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.
[57] D. L. Boger, S. Teramoto, H. Cai, *Bioorg. Med. Chem.* **1996**, *4*, 179.
[58] D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381.
[59] D. Seebach, J. V. Schreiber, S. Abele, X. Daura, W. F. van Gunsteren, *Helv. Chim. Acta* **2000**, *83*, 34.
[60] D. Seebach, T. Sifferlen, P. A. Mathieu, A. M. Häne, C. M. Krell, D. J. Bierbaum, S. Abele, *Helv. Chim. Acta* **2000**, *83*, 2849.
[61] D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1997**, *80*, 2033.
[62] D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, *Helv. Chim. Acta* **1998**, *81*, 932.
[63] T. Etezady-Esfarjani, C. Hilty, K. Wüthrich, M. Rueping, J. Schreiber, D. Seebach, *Helv. Chim. Acta* **2002**, *85*, 1197.
[64] A. Glättli, X. Daura, D. Seebach, W. F. van Gunsteren, *J. Am. Chem. Soc.* **2002**, *124*, 12972.
[65] T. Hintermann, D. Seebach, *Synlett* **1997**, 437.
[66] J. L. Parsons, H. J. Klosterman, J. L. Ninnemann, *Antimicrob. Agents Chemother.* **1967**, 415.
[67] M. Hamada, T. Takeuchi, S. Kondo, Y. Ikeda, H. Naganawa, K. Maeda, Y. Okami, H. Umezawa, *J. Antibiot.* **1970**, *23*, 170.
[68] S. Kondo, S. Shibahara, S. Takahashi, K. Maeda, H. Umezawa, M. Ohno, *J. Am. Chem. Soc.* **1971**, *93*, 6305.
[69] S. I. Harik, M. D. Hollenberg, S. H. Snyder, *Nature* **1974**, *249*, 250.
[70] R. H. Yamada, Y. Wakabayashi, A. Iwashima, T. Hasegawa, *Biochim. Biophys. Acta* **1985**, *831*, 82.
[71] S. Tanase, B. M. Guirard, E. E. Snell, *J. Biol. Chem.* **1985**, *260*, 6738.
[72] C. C. Porter, L. S. Watson, D. C. Titus, J. A. Totaro, S. S. Byer, *Biochem. Pharmacol.* **1962**, *11*, 1067.
[73] H. W. Shao, Y. Wu, R. Li, *Synth. Commun.* **2000**, *30*, 1911.
[74] T. Hayashi, A. Yamamoto, Y. Ito, E. Nishioka, H. Miura, K. Yanagi, *J. Am. Chem. Soc.* **1989**, *111*, 6301.
[75] Y. Hamada, M. Shibata, T. Sugiura, S. Kato, T. Shioiri, *J. Org. Chem.* **1987**, *52*, 1252.

Received September 11, 2003