CD Spectra in Methanol of β-Oligopeptides Consisting of β-Amino Acids with Functionalized Side Chains, with Alternating Configuration, and with Geminal Backbone Substituents – Fingerprints of New Secondary Structures?

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Dedicated to Professor *Richard Neidlein* on the occasion of his 70th birthday

β-Hexa-, β-hepta-, and β-nonapeptides, **1**–**6**, which carry functionalized side chains (CO_2R, CO_2^-, $(CH_2)_4NH_3^+$, $CH_2-CH=CH_2$) consisting of β^3-amino-acid residues of alternating configuration, or which carry geminal substituents in the 2- or 3-positions of all residues, have been synthesized (*Schemes 1–3*), and their CD spectra in MeOH are reported (*Figs. 2–6*). Strong *Cotton* effects ($\Theta > 10^5$) are indicative of the presence of chiral secondary structures. It is suggested by simple modelling (*Fig. 1*) that the new β-peptides should not be able to fold to the familiar 3_{14}-helical structures. Still, three of them (**3**, **4**, and **5**) give rise to CD spectra matching those of β-peptides that are known to be present as (*M*)- or (*P*)-3_{14}-helices in MeOH solution. While possible folding motifs (*Figs. 3,b*, and *7*) of the new β-peptides have been identified in crystal structures, an interpretation of the CD spectra has to be postponed until NMR solution structures become available. A list of all β-peptides giving rise to CD spectra with a minimum near 215 nm is included (*Table*).

1. Introduction. – Circular dichroism (CD) spectroscopy is a useful tool for analyzing structures of peptides and proteins consisting of α-amino acids (α-peptides) [1]. For some β-peptides, built entirely of β-amino acids, certain patterns of the CD spectra have also been correlated with secondary structures, such as 3_{14}, 2.5_{12} and *12/10* helices, and hairpin turns [2–4]. The CD spectra of other β-peptides, such as the oligomers of three different proline analogs [5][6], have been published, but the secondary structures (formed without H-bonding) have not been determined experimentally and can be considered unknown[5]). This is also true for β-peptidic polymers derived from the only proteinogenic β-amino acid, aspartic acid[6])[7]).

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[1]) Postdoctoral Fellow at ETH-Zürich (1998–2000).

[2]) Part of the Master's Thesis of *P. M.* and *D. B.*, ETH-Zürich (1999).

[3]) Part of the projected Ph.D. Thesis of *P. M.*, *A. H.*, and *D. B.*, ETH-Zürich.

[4]) Part of the Ph.D. theses of *C. K.* and *S. A.* (Dissertations No. 13513 and 13203, ETH-Zürich, 2000 and 1999, resp.).

[5]) Modelling of the structure of β^3-HPro oligomers suggests that they may form helices, with ten residues forming three pitches [5].

[6]) Extensive modelling has produced several helical structures, which are supported by fiber-X-ray diffraction data [7].

[7]) For review articles in which CD spectra of β-peptides are discussed, see [8][9].

Furthermore, it is expected [10][11] that β-peptides with certain substitution patterns might not be able to form the hitherto known secondary structures (*Fig. 1*).

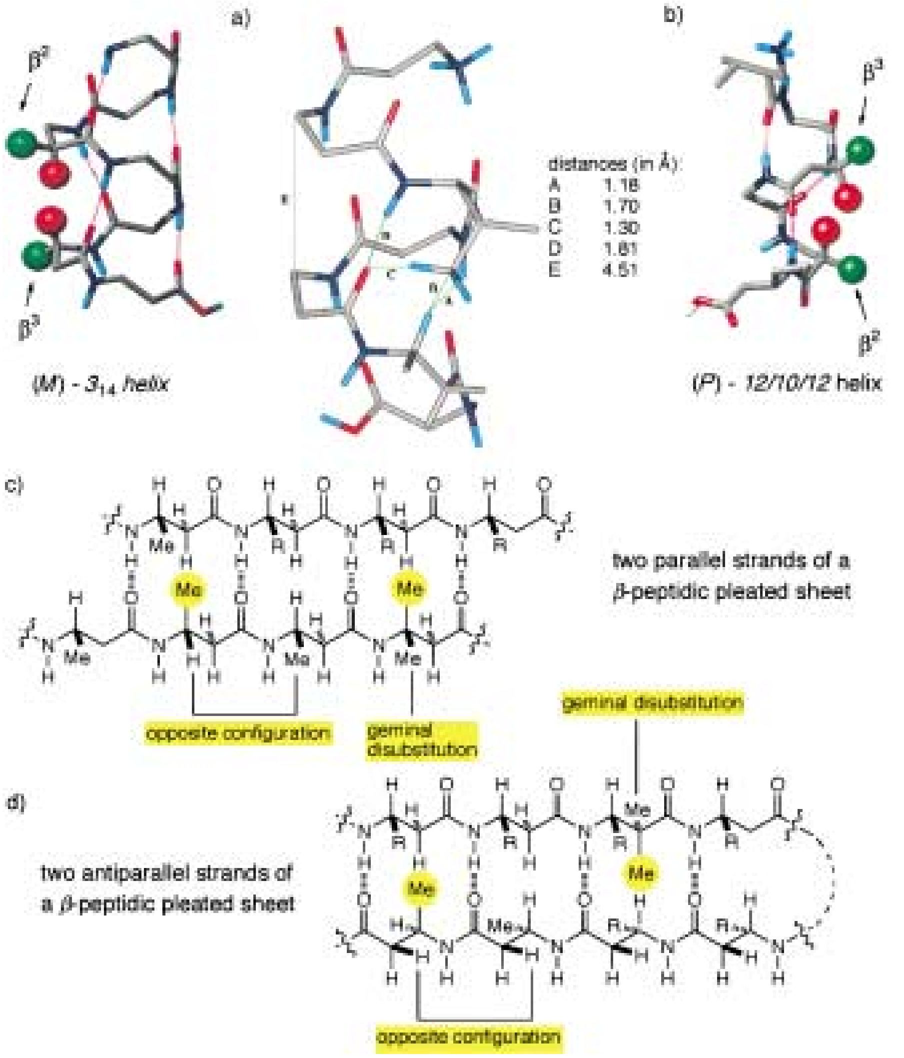

Fig. 1. *Models of β-peptidic helices and pleated sheets with alternating configuration and with geminal disubstitution of residues. a*) The (*M*)-3_{14} helix with axial substituents in 2- or 3-position of residues. The pitch of the helix is *ca*. 4.5 Å (taken from one of the NMR structures [11]). The distances between the H-atoms of a Me group placed in an axial position and atoms on the backbone one pitch upwards (A and C are 1.2–1.3 Å) are clearly shorter than the sum of the *van der Waals* radii. Alternating configuration of the residues in all-β^2- or all-β^3-peptides, or geminal disubstitution on β-amino-acid residues would create such impossible juxtapositions. *b*) Axial-type substituents in the *12/10* helix [11][12] also create severe *van der Waals* repulsions. *c*) and *d*) Pleated sheets of β-peptides. Inside the 14-membered *inter*-strand rings [3][13] held together by two H-bonds, there is no room for substituents other than H. The transannular (C,C) distance in the linear arrangement $C-CH_3\cdots H-C$ would be *ca*. 4.6 Å. Therefore, strands with alternating configuration or geminal disubstitution of β-amino-acid residues are not expected to form sheets.

Thus, the juxtaposition at *ca*. 4.5-Å distance of lateral charged substituents such as CO_2^- in an (idealized) β-peptidic 3_{14} helix may not be possible due to *Coulomb* repulsion. Also, axial substituents other than the H-atoms, or possibly Me groups, are forbidden on this 3_{14} helix, and probably also on the *12/10* helix, due to *van der Waals* repulsion. Finally, there is no room for backbone substituents inside the 14-membered H-bonded rings present in pleated sheets, neither in the world of α-peptides (Aib is sheet-breaking and helix-favoring) nor in the realm of β-peptides (*Fig. 1*). In a first step towards probing these structural considerations, we have now measured the CD spectra of β-peptides with the corresponding backbone substitution patterns.

2. CD Spectra of New β-Oligopeptides. – We have synthesized (see *Chapt. 3*) the β-peptides **1**–**6** (*Figs. 2–6*), consisting of up to nine (chiral[8])) residues. None of them has, so far, crystallized to allow for X-ray crystal-structure analysis. We have also not been able to derive a solution structure from NMR measurements, in spite of intense efforts expended on some of them. This is, at least partially, due to the fact that the homo-β-peptides (consisting of identical residues) do not give rise to sufficient dispersion of the NMR signals[9]), even with an 800-MHz instrument[10]). Furthermore, there is less $^1H,^1H$-coupling information along the backbone of β-peptides consisting of geminally disubstituted β-amino acids. Thus, we have so far only the CD spectra shown in *Figs. 2–6* providing hints from the observed *Cotton* effects as to whether or not there are secondary structures present in methanol solution, and whether or not they may be similar to those secondary structures of β-peptides (consisting of non-cyclic residues) that we have discovered previously. With two exceptions (**1c** in *Fig. 2*), all CD spectra were measured of 0.2 mM solutions in MeOH.

2.1. *Oligo-β-aspartates.* β-Peptidic oligomers of the one and only proteinogenic β-amino acid, aspartic acid, have been synthesized by *Kajtár et al.*, but the announced results of chiroptical measurements were never published [15]. *Yuki et al.* have studied stretched films and solutions of poly(α-alkyl-β-aspartates) by X-ray diffraction, IR, CD, ORD, and ^{1}H-NMR methods; they proposed a sheet-like structure in films, and observed a dramatic change of the CD spectra upon addition of H_2SO_4 [16]. In contrast, *Fernández-Santín* and *Muñoz-Guerra* and co-workers found helical structures for poly(α-alkyl-β-aspartates) by fiber X-ray diffraction, IR, CD, NMR, and computational methods [7][17–24]. They proposed three right-handed helices, with 3.25, 4, or 4.25 amino acid residues per turn, also depending upon the type of side-chain ester group CO_2R[11]).

Of the β-hexa- and β-nona-aspartates **1**, we have not obtained crystals for X-ray analysis, and NMR measurements did not provide enough dispersion and NOE information for structural assignment[10]). The CD spectra of the esters **1a** and **1b** (*Fig. 2*) were measured in 0.2 mM MeOH solution, of the ester **1d** in 0.1 mM solution,

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[8]) For geminally disubstituted achiral β-peptides with structural modelling, see [14].

[9]) Experiments by *K. Gademann* and *B. Jaun* with **2**, ETH-Zürich, 1999. For details, see Dissertation of *S. Abele*[4]).

[10]) Unpublished experiments carried out with **1** by *Per I. Arvidsson* and his colleagues at the University of Gothenburg.

[11]) The helix with 3.25 residues per pitch is actually identical with our helix designated 3_1 (crystallographic nomenclature) or 3_{14} (protein nomenclature) (*cf. Fig. 1*).

while the spectrum of the hexa(carboxylic acid) **1c** was measured in *ca.* 0.2 mM aqueous buffer (0.1M $NaHCO_3$) at pH 11. The CD spectra of the *t*-Bu esters **1a** and **1b** have a maximum at 214 nm, with **1b** showing a zero-crossing at 202 nm, a pattern similar to that reported for poly(α-isobutyl-β-aspartates) [16][26]. The molar ellipticity at 214 nm rises over-proportionally (increases per amino acid residue) going from the hexamer **1a** to the nonamer **1b**. This effect indicates a more stable secondary structure of the longer β-peptide **1b**. Similar CD spectra with a single maximum between 215 and 220 nm have been assigned to the 3_{14} helix of β-peptides consisting of (*R*,*R*)-2-aminocyclohexanecarbonyl moieties [25][27]. The heptamethyl ester **1d** shows a different spectrum with a single, weak maximum at 202 nm[12]). The hexa(carboxylic acid) **1c** gives rise to a completely different (low-intensity) CD spectrum in alkaline aqueous solution: a maximum at 228 nm and a zero-crossing at 218 nm (*Fig. 2*). This

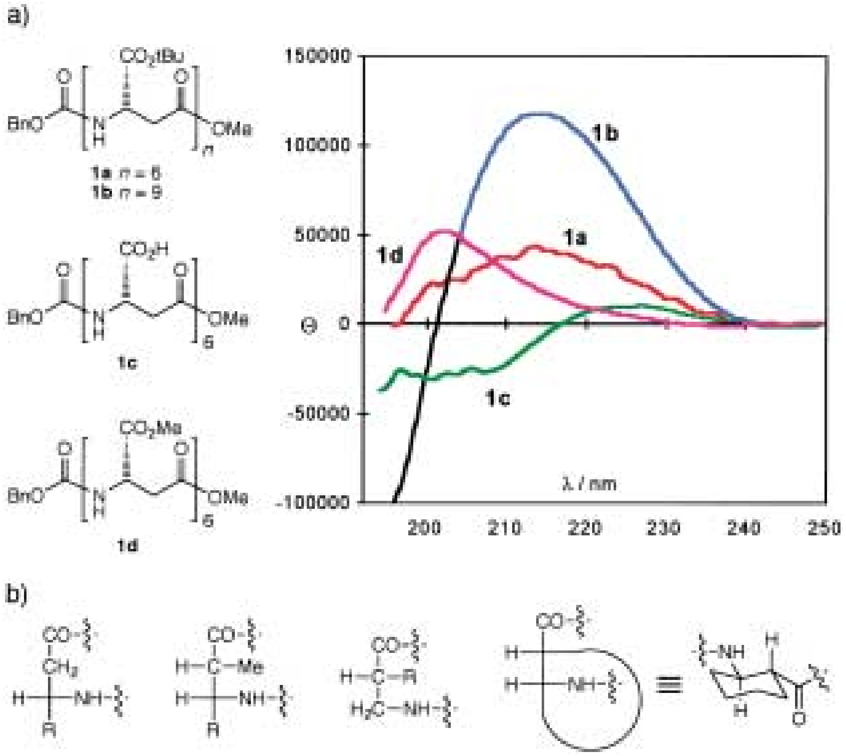

Fig. 2. *CD Spectra* (0.2 mM in MeOH for **1a** and **1b**, and 0.1 mM in MeOH for **1d**) *of β-peptides* **1** *consisting of aspartic acid, of* tert-*butyl aspartate, or of methyl aspartate. a*) The spectrum of the hexa(carboxylic acid) was measured in alkaline aqueous solution (see accompanying text); it was used after lyophilization, without further purification. The positive *Cotton* effect at *ca.* 214 nm would be compatible with a (*P*)-3_{14}-helix as formed by β^3-peptides consisting of β-amino-acid residues, *Fischer* projections of which are shown in *b*) [11][25] (note that in this presentation L- or (*R*)-aspartate would be assigned D-configuration!). For CD spectra of the so-called poly(α-alkyl-β-aspartates), see [16][26].

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[12]) The CD spectra of β-peptides containing ten-membered H-bonded rings also show a single maximum between 200 and 210 nm [2].

different pattern might be considered to indicate a secondary structure different from those of the esters: the oligo acid is expected to be fully deprotonated under these conditions (see *Chapt. 1*). We should, however, not forget that the ester groups in the side chains may influence the CD spectrum, because they are chromophores in their own right in the wavelength range of the observed *Cotton* effects[13]).

2.2. *β^3-Heptapeptides with Alternating Configuration*[14]) *of the Residues.* We next consider the CD spectra of β-peptides **A**[15]), **2a**, and **2b**, all carrying the side chains (of Ala, Val, Leu, Phe, and Lys) in the 3-position of their building blocks. Of these, only **A** is able to fold into the familiar 3_{14} helix, according to the considerations in *Chapt. 1.* Indeed, a MeOH solution of this β-nonapeptide shows the expected CD pattern ($\Theta - 4 \cdot 10^4$ at 220 nm and $+1.6 \cdot 10^5$ at 200 nm, *Fig. 3*, *a*).

The compounds **2a** and **2b** with alternating configuration of the residues would have substituents larger than H or Me in axial positions of a 3_{14} helix, be it of (*P*) or (*M*) helicity. Indeed, the CD spectrum of **2a** exhibits only one strong (negative) *Cotton* effect ($\Theta - 1 \cdot 10^5$) at 202 nm (apart from a very weak maximum at 223 and a shoulder near 210 nm), while that of **2b** could be called an indiscriminate scribbling.

Whatever the breakdown of the intensive *Cotton* effect upon replacement of two Leu side chains by Lys in the 3- and 7-positions of **2a** (→ **2b**) means, the CD spectrum of **2a** would commonly be interpreted as resulting from a chiral secondary structure. An NMR analysis[9]) led to assignment of all protons; an antiperiplanar arrangement of NH and H–C(3) in each residue is indicated by the large coupling constants (8–9 Hz) between these protons; the ROESY spectrum provided nuclear-*Overhauser* effects (NOEs) between NH or residues *i* and H–C(3) or residues (*i* – 1); all attempts to arrive at a secondary structure by simulated annealing, using these NMR data, were unsuccessful, and we tend to believe that a random coil might be present in the MeOH solution of β-heptapeptide **2a**.

Information about a possible geometry of the backbone of β^3-peptides with alternating configuration of the residues may be obtained from the crystal structure of the β-dipeptide shown in *Fig. 3*, *b*[16]). It is, however, not possible at this point to judge whether the meander-type backbone arrangement deduced from this dimer structure is an 'artefact' of the solid state, with interstrand H-bonded 14-membered rings, or whether it is also present in solutions of longer-chain analogs such as the β-peptides **2**.

2.3. *β-Hexapeptides Built of the Geminally Disubstituted $\beta^{2,2}$-, $\beta^{3,3}$-, and $\beta^{2,2,3}$-Amino Acids.* We next tested a couple of β-peptides that could fold to a 3_{14} helix only by placing a Me group in an axial position (*cf. Fig. 1*).

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[13]) Optically active acids and esters, with a chirality center α to the C=O group, show *Cotton* effects in the range of 207 to 240 nm [1][28].

[14]) The term 'alternating configuration' is correct only when we use the *Fischer* convention, according to which **2a** and **2b** have (L, D, L, D, L, D, L)-configuration. According to the *CIP* convention, the assignment would be (*R*,*R*,*S*,*R*,*R*,*R*,*S*), going from N- to C-terminus, due to a change in priority in L-β-HVal. Alternatively, we could have applied the *Masamune* convention for assignment of relative configuration in the *zig-zag* projection, according to which **A** would be all-*syn* and **2a** and **2b** all-*anti*.

[15]) Compound **A** has been shown to be an inhibitor of cholesterol and lipid absorption in the small intestine [29].

[16]) The β-dipeptide derivative Boc-(*R*)-β-HAla-(*S*)-β-HAla-OBn was prepared by Dr. *M. Oberhoff*, and the X-ray crystal structure determined by *P. Seiler* (ETH-Zürich, 1997/98).

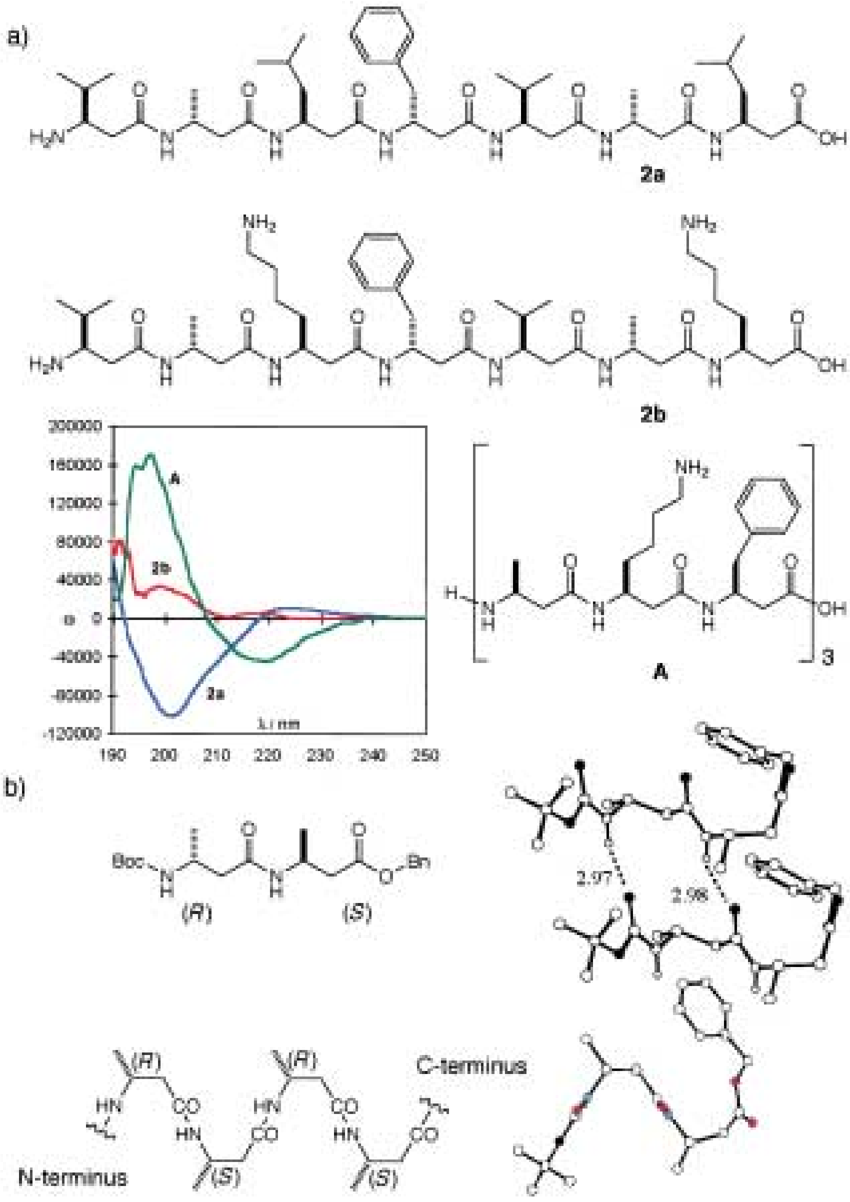

Fig. 3. *CD Spectra* (0.2 mM in MeOH) *and an X-ray crystal structure of β^3-peptides with alternating configuration of the residues. a*) Formulae of β-heptapeptides **2a** and **2b** and CD spectra in MeOH (0.2 mM), comparison with an all-(*S*)-β^3-nonapeptide **A** (exhibiting the CD pattern associated with an (*M*)-3_{14} helix). Note the collapse of the CD pattern on incorporation of two lysine side chains in (*i*)- and (*i* + 4)-positions of the β-heptapeptide **2b**. *b*) Molecular structure and packing in the crystal of Boc-(*R*)-β^3-HAla-(*S*)-β^3-HAla-OBn, and possible folding of a higher oligomer of this type.

The most simple series of hexamers **3** studied consists of identical 2,2-dimethyl-β-HAla residues ('homo-β-peptides'). It was surprising to see that the CD spectra in MeOH of the protected (**3a**) and of the unprotected (**3b**) β-peptide are almost superimposable with those of our standard β-hexapeptide [2][13] H-(β^3-HVal-β^3-HAla-β^3-HLeu)$_2$-OH **B** (*Figs. 4* and *5*), with a negative *Cotton* effect at 215 and positive one at 196 nm; only the intensities differ somewhat. Even with one 'wrong' β-amino acid of (R)-configuration (**3c**) in position 4 of the hexapeptide do we observe the familiar CD pattern, albeit of clearly reduced intensity of the minimum and of the maximum[17]). With alternating (S,R,S,R,S,R) configuration of the six residues (**3d**) there is a total breakdown of the circular dichroism[18]). Also, introduction of two geminal Me groups in the parent hexapeptide **B** ($\rightarrow$ **4**) leads to a spectrum (*Fig. 5*) with the familiar shape, but of reduced intensity of the *Cotton* effect at 213 nm from Θ *ca.* $-4 \cdot 10^5$ to $-2 \cdot 10^5$.

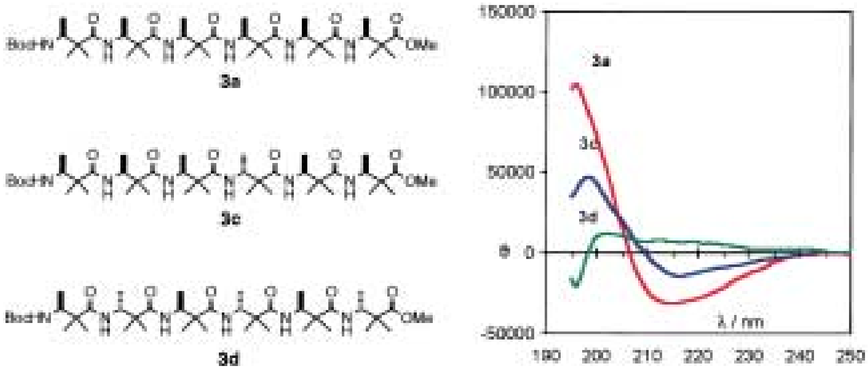

Fig. 4. *CD Spectra* (0.2 mM in MeOH) *of protected* β-*hexapeptides* **3a**, **3c** *and* **3d** *carrying three Me groups on each residue* (2,2-Me$_2$-β-HAla of (R)- or (S)-configuration). For the free acid **3b**, see *Fig. 5*. The compounds **3a** and **3b** with all-(S)-configuration exhibit the trough/zero-crossing/peak CD pattern typical of β^3-peptides lacking the geminal Me groups, and so does compound **3c** with a residue of 'wrong' (R)-configuration in position 4. There is a breakdown of the *Cotton* effects with alternating configuration of the residues as in **3d** (*cf. Fig. 3*).

Another series of β-peptides with geminally disubstituted residues are the $\beta^{2,2}$- and the $\beta^{3,3}$-hexapeptides **5** and **6**, respectively. The CD spectra of the fully protected (**5a** and **6a**), of the N-Boc-protected (**5b** and **6b**), and of the unprotected compounds (**5c** and **6c**) are shown in *Fig. 6*.

The spectrum of the $\beta^{2,2}$-hexapeptide **5** (essentially superimposable traces for all three derivatives **a**, **b**, and **c**) is again surprising: it has the typical pattern [11][30] – of weak intensity – associated with (P)-3_{14}-helical β-peptidic structure (mean values for Θ

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[17]) With the simple β-hexapeptide H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**B**), the introduction of a seventh central β^3-amino acid of 'wrong' (R)-configuration, *i.e.*, Boc-β-HVal-β-HAla-β-HLeu-(R)-β-HAla-β-HVal-β-HAla-β-HLeu-OMe gives rise to a complete breakdown of the '3_{14}-helical' CD spectrum.

[18]) This is in contrast to the situation encountered with the β^3-peptides lacking the 2,2-dimethyl groups: alternating configuration of the residues (with lipophilic side chains) gives rise to a new, quite distinctive CD spectrum as compared to that of (isomeric) β^3-peptides with homochiral residues (compare **2a** in *Fig. 3* with **B** in *Fig. 5*).

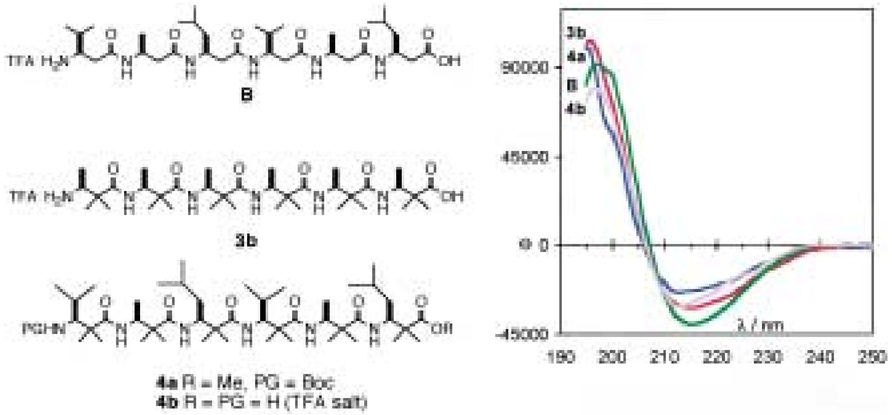

Fig. 5. *Comparison of the CD spectra* (0.2 mM in MeOH) *of H-(β^3-HVal-β^3-HAla-β^3-HLeu)$_2$-OH* (**B**) *with those of the analogs carrying two Me groups in the 2-position of each residue* (**4b**) *and of H-(2,2-Me$_2$-β-HAla)$_6$-OH* (**3b**)

and λ: $+2.7 \cdot 10^5$ at 220 and $-2.2 \cdot 10^5$ at 202 nm); in fact, we would have expected to see such a spectrum for the analogous compound lacking the Me groups (the hexademethyl derivative of **5**, an all-(R)-β^2-hexapeptide)! The $\beta^{3,3}$-geminally disubstituted residues in **6** lead to a totally different effect: the CD spectrum below 225 nm consists of an all-positive curve with a shoulder at *ca.* 215 nm (Θ $+3 \cdot 10^4$) and an intensive maximum at 197 nm (Θ $+1.3 \cdot 10^5$). Thus, this spectrum does not resemble the pattern of any other CD spectrum that has previously been assigned to a known β-peptidic secondary structure[19]). With β-peptides consisting of achiral geminally disubstituted residues, we have identified by X-ray crystal-structure analysis two H-bonding motifs, a ten-membered ring (oligomers of (aminomethyl)cyclopropanecarboxylic acid [14]) and a twelve-membered ring (oligomers of (aminomethyl)cyclohexanecarboxylic acid [31]; see *Fig. 7*). While we have no way of knowing how the CD spectrum of a chiral oligomer built of the eight-membered H-bonded rings (shown in *Fig. 7, a*) might look[20]), we have correlated an intensive *Cotton* effect near 200 nm to a ten-membered-ring motif (of the type pictured in *Fig. 7, b*) built of a β^2/β^3-dipeptide sequence [2][11][12].

2.4. *Conclusion from the CD Analysis.* The spectra reported herein are surprising: *i*) Methoxycarbonyl and (*tert*-butoxy)carbonyl side chains on a β-peptidic backbone give rise to different CD spectra (*Fig. 2*). *ii*) Alternating configuration of the residues in a β^3-peptidic backbone may (**2a**) or may not (**2b**) lead to an intensive *Cotton* effect, depending upon the absence or presence of two lysine side chains in 3- and 7-positions of a β-heptapeptide (*Fig. 3*). *iii*) Replacement of the two H-atoms (CH_2) by two Me groups (Me_2C) in the 2-position of the residues in a β^3-peptide does not cause a change

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[19]) If we would consider **6** a β^3-peptide, with the α-H-atoms replaced by Me groups, we would have expected the pattern of a (P)-3_{14}-helical CD spectrum, in analogy to the results obtained with the $\beta^{2,2}$-peptide **5** (see, however, the modelling in *Fig. 1*).

[20]) The aminooxy-acid oligomers reported by *Yang et al.* also fold to eight-membered H-bonded rings [32][33]; their optical properties can, however, probably not be compared with those of our β-peptides.

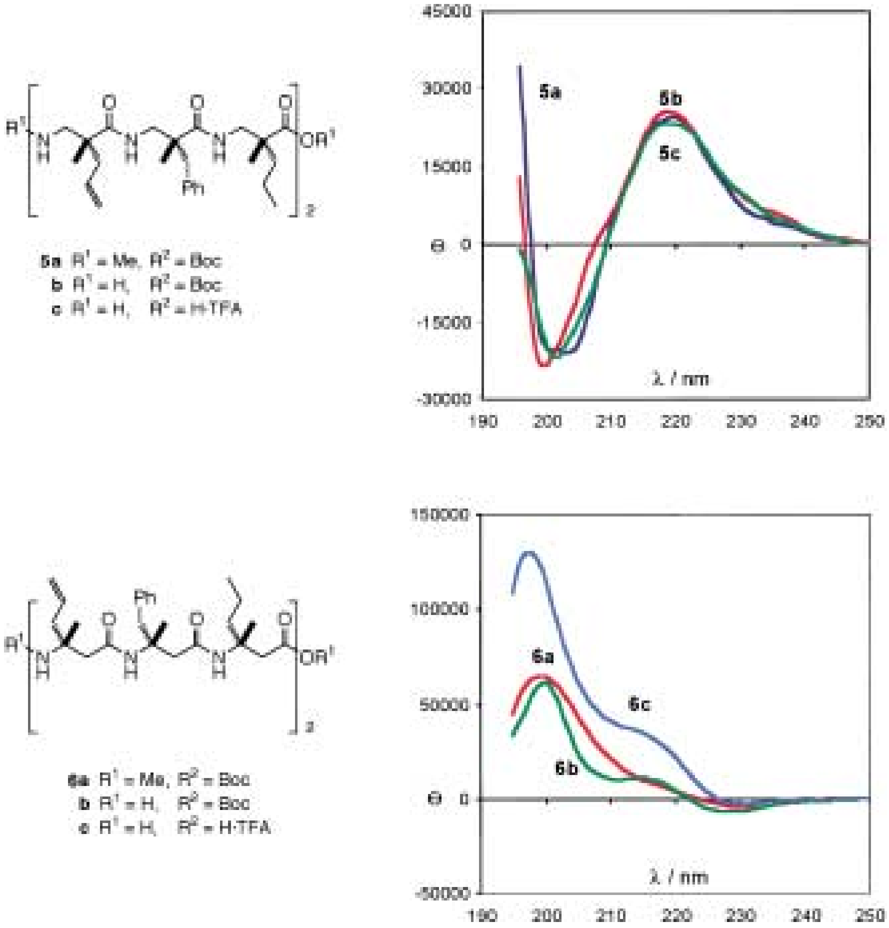

Fig. 6. *CD Spectra* (0.2 mM in MeOH) *of $\beta^{2,2}$- and $\beta^{3,3}$-hexapeptides* **5** *and* **6** *carrying geminal Me/allyl, Me/Bn, and Me/Pr groups in 1,3-, 2,5-, and 3,6-positions, respectively, of their all-(*R*)-residues.* The $\beta^{2,2}$-peptide **5** gives rise to a peak/zero-crossing/trough CD pattern, which we would associate with a (*P*)-3_{14}-helical secondary structure of a β-peptide built of monosubstituted residues. The $\beta^{3,3}$-isomer **6** shows a completely different CD pattern (230 nm trough/215 nm peak/200 nm peak)

of pattern of the CD spectrum (*Figs. 4* and *5*). *iv*) Upon replacement of the H-atom at C(2) by a Me group in the residues of a β^2-peptide, the CD peak and trough remain, while the same change in a β^3-peptide leads to a new CD pattern (*Fig. 6*).

In view of our expectations mentioned in the *Introduction* (*Fig. 1*), these results are puzzling, to say the least. A comparison (see *Table*) of β-peptides with very different primary structures and certainly also different secondary structures, would suggest that there is not much variety among their CD spectra! Thus, there is not much valuable structural information in these CD spectra. Actually, they create strong motivation for us to try very hard to determine the structures of our novel β-peptides by other methods.

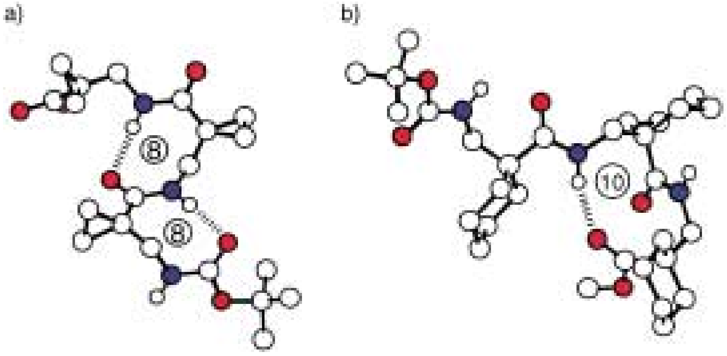

Fig. 7. *Two H-bonded rings identified in X-ray crystal structures of $\beta^{2,2}$-oligopeptides carrying dimethylene* [14] *and pentamethylene* [31] *(geminal) substituents in the 2-position of their residues.* The eight- and ten-membered H-bonded rings (*a* and *b*, resp.) might be present in the solution structures of β-peptides **3**, **4**, **5**, and **6** reported herein.

3. Comments on the Synthesis of *β*-Peptides 1–6. – All peptides, of which the CD spectra are presented herein, as well as the new β-amino-acid building blocks and the synthetic intermediates on the way to the oligomers have been fully characterized by their IR, NMR, and CD spectra, and by elemental analysis or high-resolution mass spectrometry. The unprotected β-peptides **A**, **B**, **2a/b**, **3b**, **4b**, **5c**, and **6c** have all been purified by preparative reversed-phase high-performance liquid chromatography (RP-HPLC). Full details will be reported as soon as we are able to present also NMR-solution, NMR-solid-state, or X-ray crystal structures. Thus, only a brief outline of how we prepared the β-amino acids and synthesized the β-peptides therefrom is given below (see *Schemes 1–3*). For a complete collection (as of mid 1999) of the known methods for the preparation of geminally disubstituted β-amino acids, see our recent review article [35].

3.1. *β^3-Peptides from* L*-Aspartic Acid.* The suitably protected monomeric building blocks were prepared by known procedures [36–38]. The β^3-homopeptides **1a** and **1b** were synthesized in solution by standard coupling methods (EDC/HOBt)[21]) [10][11][13] (*Scheme 1*).

The *t*-Bu ester groups of the β^3-hexapeptide **1a** were cleaved in a CF_3COOH (TFA)/H_2O mixture to give the hexa(carboxylic acid) **1c**. The heptamethyl ester **1d** was prepared from **1c** with CH_2N_2 and purified by preparative RP-HPLC.

3.2. *β^3-Peptides with Alternating Configuration.* *N*-Fmoc-β^3-Amino acids were obtained from the corresponding α-amino acids by *Arndt-Eistert* homologation as described in the literature [39–42]. The synthesis of the β^3-heptapeptides **2a** and **2b**, as well as of the β^3-nonapeptide **A**[15]), was performed on *ortho*-chlorotrityl-chloride resin [43]. Peptide **2a** was cleaved from the resin by 2% TFA in CH_2Cl_2, while TFA/H_2O/(i-Pr)$_3$SiH was used for **2b**, containing acid-labile protecting groups in its functionalized side chains.

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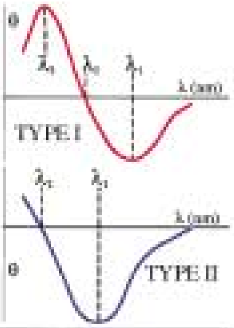
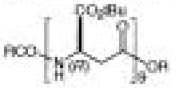
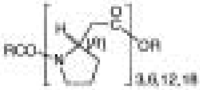
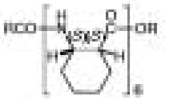
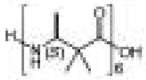
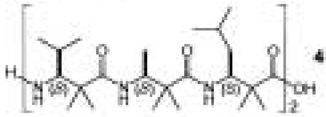
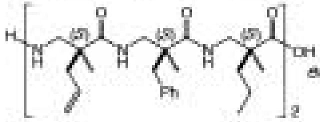
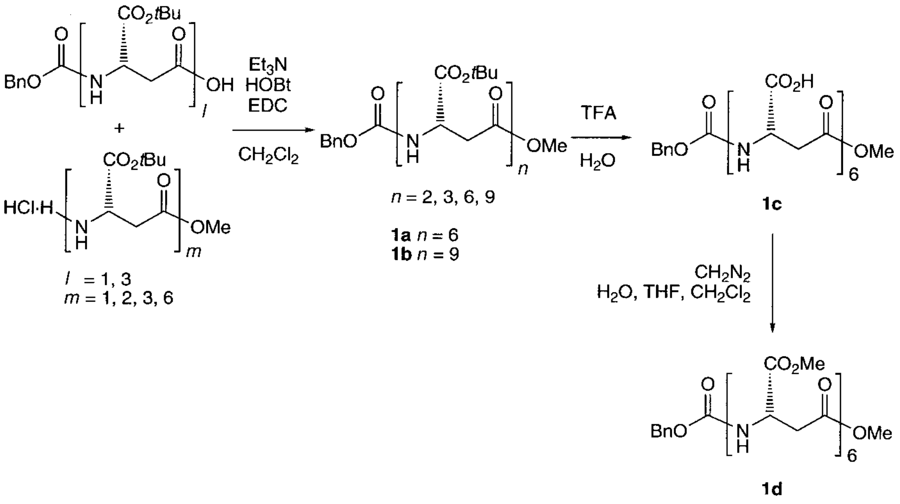
[21]) EDC = *N*-[3-(Dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride, HOBt = 1-hydroxy-1*H*-benzotriazole.

Table. *CD Patterns of Various β-Peptides* (MeOH solutions). *Type-I* CD spectra exhibit a negative *Cotton* effect between 229 and 214 nm (λ_1), a zero-crossing between 206 and 223 (λ_2), and a positive *Cotton* effect between 210 and 196 nm (λ_3). *Type-II* CD spectra present only the longer-wavelength minimum (λ_1), no maximum, and, in one case not even a zero crossing (λ_2) (–). For comparison purposes, we show in some cases the enantiomers and the mirrored CD spectra of those actually investigated and measured, respectively. Three of the β-peptides have been shown by NMR analysis to fold in MeOH to an (*M*)-*3*$_{14}$ helix, one to an (*M*)-*2.5*$_{12}$ helix. The other seven β-peptides (?) have unknown secondary structures, and, most likely, can not fold to *3*$_{14}$ or *2.5*$_{12}$ (or *12*/*10*) helices (see *Fig. 1*).



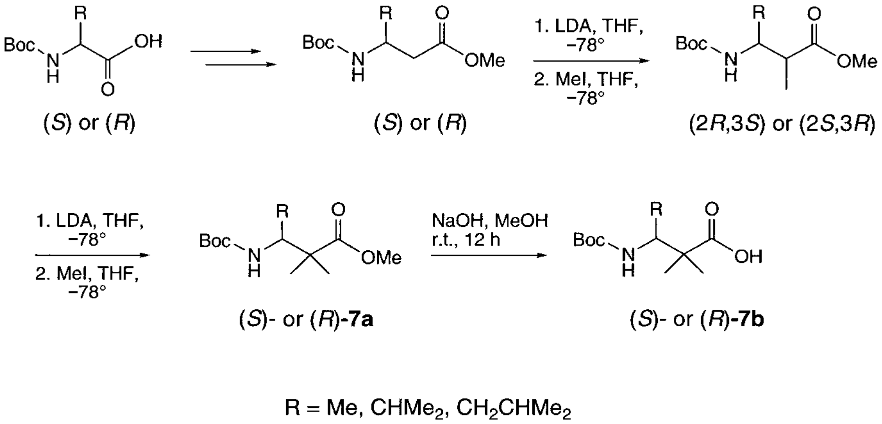
β-Peptides	References	Type	λ_1 [nm]	λ_2 [nm]	λ_3 [nm]	NMR Structure (in MeOH)
H-(β³-HVal-β³-HAla-β³-HLeu)₂-OH (**B**)	[2] [30]	I	216		200	(*M*)-*3*$_{14}$ helix
all-(*S*)-H-β³-HVal-β³-HAla-β³-HLeu- (*S*,*S*)-2Me-β-HAla-β³-HVal-β³-HAla-β³-HLeu-OH	[11]	II	215	204	–	(*M*)-*3*$_{14}$ helix
 *ent*-**1b**	this paper	II	214	202	–	?
	[5]	I	223	213	203	?
	[5] [6]	I	226 229	220 223	206 209	?
	[6]	II	215	203	–	?
	[34]	I	222	214	204	(*M*)-*2.5*$_{12}$ helix
	[25]	II	218	–	–	(*M*)-*3*$_{14}$ helix
 **3b**	this paper	I	215	207	196	?
 **4b**	this paper	I	214	206	198	?
 *ent*-**5c**	this paper	I	218	209	202	?

Scheme 1



3.3. *$\beta^{2,2,3}$-Amino-Acid Building Blocks* **7** *and Coupling to the β-Peptides* **3** *and* **4**. For constructing the $\beta^{2,2,3}$-peptides, the $\beta^{2,2,3}$-amino-acid derivatives (*S*)-**7** and (*R*)-**7** with the side-chains of alanine, valine, and leucine had to be prepared in enantiomerically pure form. To this end, the *N*-Boc-protected β^3-amino acid methyl esters, obtained from the corresponding Boc-protected α-amino acids by *Arndt-Eistert* homologation [39–42], served as starting materials (*Scheme 2*).

Scheme 2

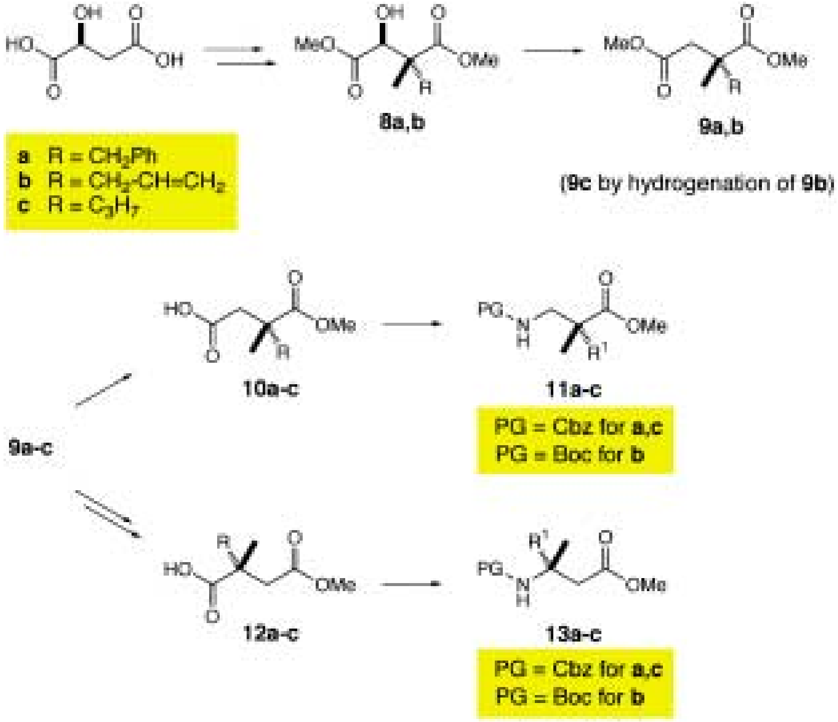


Methylation of Boc-β^3-HAla-OMe, Boc-β^3-HVal-OMe, and Boc-β^3-HLeu-OMe, or their enantiomers through doubly lithiated species provided mixtures of Boc-protected *u*- and *l*-2-methyl-3-aminoalkanoates [11][44][45], which were subjected to a second methylating cycle[22]) to give the geminally dimethylated *N*-Boc-protected methyl esters **7a**, saponification of which yielded the Boc-protected $\beta^{2,2,3}$-amino acids **7b**.

The β-peptides **3** that carry only Me groups on the backbone were obtained by the conventional coupling method (EDC/HOBt) in solution. The β-peptide **4** with leucine and valine side chains required HATU[23]) instead of EDC as the coupling reagent.

3.4. N-*Boc- and* N-*Cbz-Protected* $\beta^{2,2}$- *and* $\beta^{3,3}$-*Amino-Acid Derivatives* **11** *and* **13** *and Coupling to the* β-*Peptides* **5** *and* **6**. – The $\beta^{2,2}$- and the $\beta^{3,3}$-amino acids required for the construction of β-hexapeptides **5** and **6** were prepared from (−)-(*S*)-malic acid according to a method for alkylation we developed many years ago [47–50]. Thus, dimethyl malate was doubly alkylated diastereoselectively; in the key step of the synthesis of both, the chiral $\beta^{2,2}$- and $\beta^{3,3}$-amino-acid derivatives **11** and **13** were formed (*Scheme 3*).

Scheme 3



The malate was first methylated, and the *ca.* 9:1 mixture of diastereoisomers was directly employed for a benzylation or an allylation to give the dialkylated products **8a** and **8b** with diastereoselectivities above 93% (by ^{1}H-NMR analysis)[24]). The geminally

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[22]) One of the diastereoisomers from the first methylation was deprotonated much more slowly!

[23]) *N*,*N*,*N'*,*N'*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate [46].

[24]) The diastereoisomers could not be separated by flash chromatography; hence, all following steps were carried out using the mixtures, and separation was postponed until a later stage.

dialkylated malates were converted to the chloro diesters by *Appel* reaction and reduced to the corresponding succinates **9a** and **9b**, respectively, by Zn reduction. The allylated succinate **9b**, in turn, was hydrogenated to the Me/Pr derivative **9c**. The dialkylated succinates **9a – c** were converted to the *N*-protected $\beta^{2,2}$-amino acid esters **11a – c** by selective saponification (→ **10a – c**) and *Curtius* rearrangement[25]), with trapping of the intermediate isocyanate by either $PhCH_2OH$ or by *t*-BuOH[26]). The $\beta^{3,3}$-amino-acid derivatives **13** were prepared analogously, by degradation on the other end of the succinates **9** (*Scheme 3*): total saponification of the diesters **9**, selective esterification to the monoesters **12**, and *Curtius* rearrangement yielded the Cbz- and Boc-protected esters **13**.

Solution synthesis of the $\beta^{2,2}$-hexapeptide **5** was achieved by standard coupling methods, using EDC/HOBt for activation of the acids derived from the esters **11**. To obtain satisfactory yields, the $\beta^{3,3}$-hexapeptide **6** had to be built up by HATU coupling of the acids with the amino esters, as obtained from **13**[27]).

4. Conclusion. – As so often encountered in our group, synthesis is far ahead of structure determination. CD Spectroscopy turns out to provide hints about secondary structures, but, at the present stage of knowledge of *β*-peptides, it is not a tool for determining structures. As we have assumed previously [2], the CD of *β*-peptides, which fold in a non-cooperative way [53], may be dominated by contributions from single H-bonded rings, and we know neither all of those contributions nor their size. Thus, we feel like detectives having secured many pieces of evidence and lots of fingerprints, without being able to put everything together to catch and convict the villain.

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[25]) DPPA (diphenoxyphosphoryl azide) was used as the reagent [51].

[26]) Simple β^2- and β^3-amino acids have also been prepared enantioselectively by *Curtius* degradation of succinic acid monoesters, obtained by *Evans* methodology [52].

[27]) It is interesting to note that steric hindrance of the amino groups on a *tert*-C-atom (R_3C-NH_2; *cf.* **6**) is more detrimental to peptide coupling than steric effects on the reactivity of an activated carboxy group by a quaternary carbon (R_3C-COX; *cf.* **5**), at least in the present case.

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