Synthesis of a β-Hexapeptide from (*R*)-2-Aminomethyl-alkanoic Acids and Structural Investigations

Tobias Hintermann and Dieter Seebach*

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule ETH Zürich, CH-8092 Zürich, Switzerland

Fax +41-1-632 1144; e-Mail:<Seebach@org.chem.ethz.ch>

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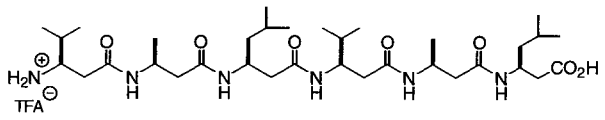
Dedicated to Professor Elias J. Corey, our teacher of organic synthesis and our model as a scientist

Abstract: The (*R*) α-branched β-amino acid derivatives **3-5** with the side chains of alanine, valine and leucine are prepared by aminomethylation of acyloxazolidinones **2a-c** and are used for the synthesis of the trifluoroacetate salt **9** of H(-β²-HVal-β²-HAla-β²-HLeu)₂-OH. The CD spectrum of compound **9** is compared with that of the isomer H(-β³-HVal-β³-HAla-β³-HLeu)₂-OH (**1**) built from the corresponding β-branched β-amino acids.

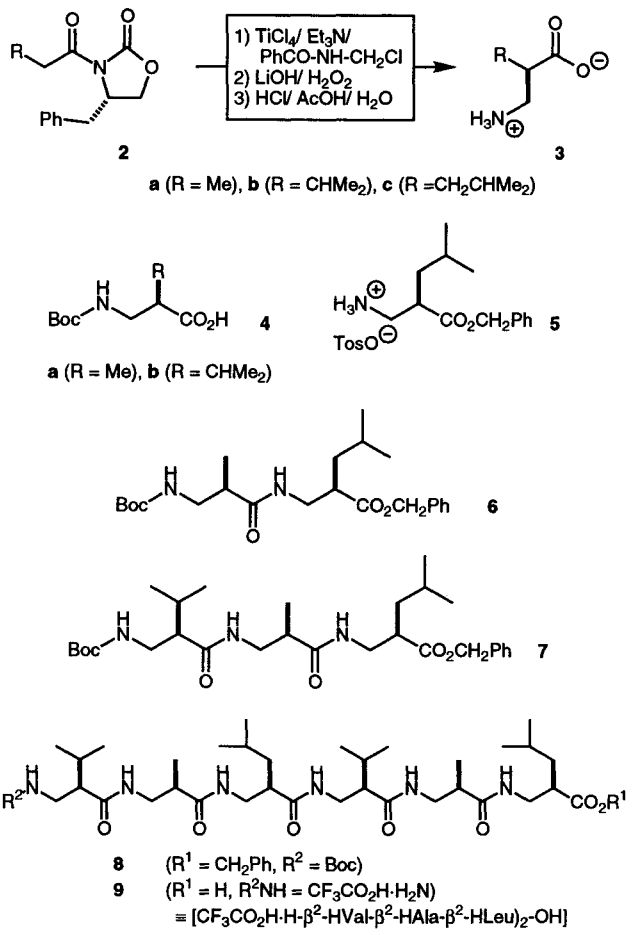
Introduction: We have previously reported the synthesis of peptides consisting of two to seven β-amino acids.[1] The β-amino acid building blocks were obtained by Arndt-Eistert homologation of L α-amino acids.[2] To the specialists and our own surprise, these short chain analogs of normal peptides showed distinct secondary structures in solution and in the solid state. Thus, the β-peptide **1** is present as a left handed or (*M*) helix in methanol solution, as determined by NMR spectroscopy (see Fig. 1a). We wondered whether an isomeric β-peptide carrying the same side chains in the α- rather than the β-position of the β-amino acid would also form a regular secondary structure, and we report here the first synthesis of such a compound (**9**) and some preliminary structural investigations. In order to distinguish between these positional isomers, we propose the terms β³-and β²-peptides (the numbers indicating the position of the side chains in the β-amino acids) for compounds of type **1** and **9**, respectively.



1 [CF_3CO_2H·H(-β³-HVal-β³-HAla-β³-HLeu)₂-OH]

Synthesis of the β²-Hexapeptide: As a straightforward access to β²-amino acid building blocks we chose *Evans'* method.[3,4] The (*S*)-phenylalanine-derived auxiliary oxazolidinone was acylated with propanoyl, 3-methyl-butanoyl and 4-methyl-pentanoyl chloride to give **2a-c**. Aminomethylation with N-chloromethyl-benzamide produced the β-amino acid derivatives with high diastereoselectivities (93:7 - 99:1) and high yields (78-85% diastereoisomerically pure product after purification). Removal of the chiral auxiliary followed by hydrolysis of the N-benzoyl group gave the free enantiopure (*R*) amino acids **3a-c** (Scheme 1).[5,6] These, in turn were protected for coupling by standard procedures[7] (Boc-β²-HAla-OH, **4a**, Boc-β²-HVal-OH, **4b**, TosOH·H-β²-HLeu-OBn, **5**). The β²-dipeptide **6** was obtained (87%) by coupling **4a** and **5** using the mixed anhydride method.[7] Boc-Deprotection (HCl/dioxane) and coupling with **4b** (EDC/HOBt)[8] produced the β²-tripeptide **7** (76%). For fragment coupling to the protected β²-hexapeptide **8**, one part of **7** was treated with HCl in dioxane, another part with H_2/Pd-C in MeOH, and the resulting tripeptide derivatives were combined using the EDC/HOBt method (69%). The free β²-hexapeptide **9** was then obtained by sequential treatment with CF_3COOH/CH_2Cl_2 and H_2/Pd-C/MeOH (Scheme 1). The β²-di-, -tri- and -hexapeptide derivatives **6-9** have been fully characterized (IR, ^{1}H-, ^{13}C-NMR, FAB-MS, $[\alpha]_D$, m.p. and, for some, elemental analysis).

Structure and CD-Spectra of **9**: As mentioned above, the β³-peptides such as **1**, built from β-amino acids of L configuration,[9] form (*M*) 3_1 helices in MeOH solution (Fig. 1a). A β-peptide built from the amino acids **3** is predicted to have the opposite (*P*) helicity as shown in Fig. 1b. As with α-peptides, the presence of a helical secondary structure of a β-peptide may be corroborated by optical measurements: In the course of our investigations on linear β³-hexa- and β³-heptapeptides, we have observed characteristic CD spectra in MeOH, with a typical pattern of a trough at *ca.* 216 and a peak at *ca.* 197 nm (*cf.* Fig. 2 (----)). The conclusion from numerous optical measurements,[10] combined with structural information from NMR spectroscopy, is that this CD pattern is indicative of the (*M*) 3_1 helix as pictured in Fig. 1a. The CD spectrum in methanol of the novel β²-hexapeptide **9**, measured at room temperature, is shown in Fig. 2 (——). Indeed, the pattern of the spectrum of **9** is similar to that of its isomer **1**, but the sign of the cotton effect (CE) is positive at 216 nm (molar ellipticity Θ [10 deg cm² mol⁻¹] = $3.3 \cdot 10^4$ vs. $-4.9 \cdot 10^4$) and negative at 198 nm (Θ = $-7.9 \cdot 10^4$ vs. $9.1 \cdot 10^4$); such mirror image-type spectra result from the two isomers (Fig. 2). This would be in accordance with **9** forming a right-handed or (*P*) helix, the backbone of which has a mirror-image type relationship with that of **1** (*cf.* Fig. 1a and b). The less intense CE of **9** compared to **1** could indicate a somewhat less stable secondary structure. In order to examine the



Scheme 1. Preparation of (*R*)-3-amino-2-methyl-propanoic acid (**3a**), of the (*R*)-2-aminomethyl-alkanoic acid derivatives **3b ,c , 4, 5**, and synthesis of the β²-hexapeptide **9**

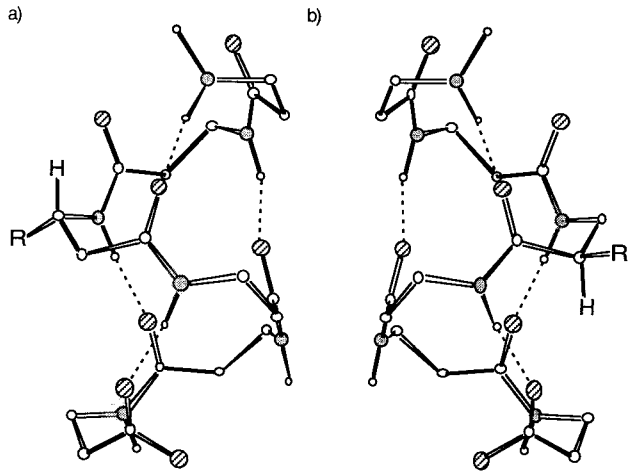


Figure 1. 3_1-Helices of β-hexapeptides. (a) Left-handed helix of **1** in MeOH as determined by NMR spectroscopy. (b) Expected right-handed helical secondary structure of a β^2-hexapeptide consisting of α-branched β-amino acids of (*R*) configuration. Only one side chain R is included in the presentations

stability, we measured the CD spectrum of **9** at -20 °C, which resulted in a 40% increase of the molar ellipticity of the 216 nm CE. More dramatic alterations occur upon solvent changes. Whereas the molar ellipticity at 216 nm decreases in water from $3.3 \cdot 10^4$ (methanol) to $1.7 \cdot 10^4$, it increases in acetonitrile to $5.9 \cdot 10^4$ (this is an even stronger CD-effect, than observed for peptide **1** in methanol). Thus, we have shown that less polar solvents favour the formation of the secondary structure of **9**. In order to confirm this interpretation of the CD results, the structure of **9** in solution will be investigated by NMR analysis.

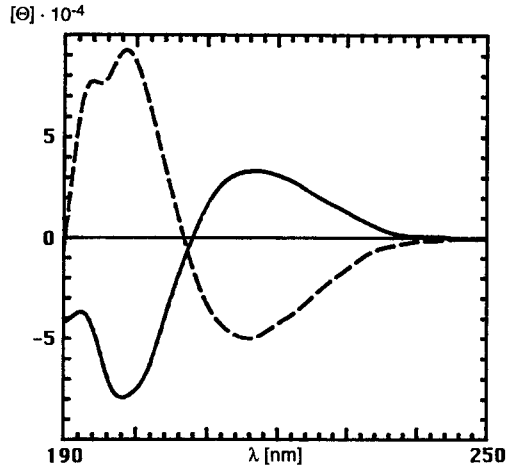


Figure 2. CD Spectra of the β^3-hexapeptide **1** (- - -) and of the β^2-hexapeptide **9** (——) in MeOH. Concentration $2 \cdot 10^{-4}$ M, molar ellipticity [Θ] in 10 deg cm^2 mol^{-1}

Stability to Enzymatic cleavage: β-Peptides as analogs of α-peptides are potential drug candidates. A major advantage of β-peptides compared to α-peptides might be that they are not degraded by proteases. We have previously shown that the β^3-hexapeptide **1** is stable towards the action of pepsin.[1] We have now also tested the new β^2-hexapeptide **9** as a substrate for pepsin and have found that it is fully stable for several days under conditions where the α-peptide H-(Val-Ala-Leu)$_2$-OH is degraded within minutes.

Characteristic Data of **8**:[11] Amorphous white solid. $[\alpha]_D$ = -110.2 (c = 0.95, $CHCl_3$). IR ($CHCl_3$): 3445*m*, 3307*m*, 3008*m*, 2965*s*, 2873*m*, 1702*m*, 1654*s*, 1522*s*, 1469*m*, 1368*m*, 1170*s*. ^{1}HNMR (500 MHz, $CDCl_3$): 0.88-0.93 (*m*, 18H, 6 CH_3); 0.96 (*d*, *J* = 6.6, 6H, 2 CH_3); 1.03 (*d*, *J* = 7.0, 3H, CH_3); 1.16 (*d*, *J* = 7.1, 3H, CH_3); 1.24-1.29 (*m*, 2H, 2 $CH_2CH(CH_3)_2$); 1.43 (*s*, 9H, $C(CH_3)_3$); 1.52-1.63 (*m*, 4H, 2 $CH_2CH(CH_3)_2$); 1.80-1.85 (*m*, 1H, $CHRCH(CH_3)_2$); 1.89-1.92 (*m*, 1H, $CHRCH(CH_3)_2$); 1.99-2.05 (*m*, 1H, COCHR); 2.06-2.11 (*m*, 1H, COCHR); 2.42-2.52 (*m*, 3H, 3 COCHR); 2.71-2.76 (*m*, 1H, COCHR); 3.05-3.75 (*m*, 12H, 6 NCH_2); 5.10 (*d*, *J* =12.3, 1H, CH_2Ph); 5.16 (*d*, *J* = 12.2, 1H, CH_2Ph); 5.54 (*t*, *J* = 5.9, 1H, NHBoc); 6.80 (br., 1H, NHCO); 7.02 (br., 1H, NHCO); 7.09 (br., 1H, NHCO); 7.15 (br., 1H, NHCO); 7.31-7.37 (*m*, 6H, 5 H-arom./ NHCO). ^{13}CNMR (125 MHz, $CDCl_3$): 15.4; 15.5; 20.3; 20.4; 21.0; 21.1; 22.3; 22.6; 23.1 (CH_3); 25.9; 26.0; 28.4; 28.4 (CH); 28.5 (CH_3); 38.8; 38.8; 39.8 (CH_2); 40.8 (CH); 41.0; 41.2 (CH_2); 42.0; 42.3; 42.6; 42.8; 44.3; 45.3; 54.8; 55.1 (CH); 66.7 (CH_2); 79.0 (C); 128.2; 128.3; 128.6 (CH); 135.9; 156.3; 174.6; 174.6; 174.9; 175.2; 175.3; 175.4 (C). MS (FAB): 860 (18, $[M+H]^+$), 859 (30, M^+), 764 (10), 761 (20), 760 (59), 759 (100), 91 (21).

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References and Notes

(1) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043.

(2) For a review see: Matthews, J. L.; Braun, C.; Guibourdenche, C.; Overhand, M.; Seebach, D. Chapter 5 in *Enantioselective Synthesis of β-Amino Acids*, Juaristi, E., Ed., VCH, Weinheim and New York, 1997.

(3) Evans, D. A.; Urpi, F.; Somers, T. C.; Clark, S. C.; Bilodeau, M. T. *J. Am. Chem. Soc.* **1990**, *112*, 8215.

(4) For alternative methodologies, see the monograph referred to in (2) and: Juaristi, E.; Quintana, D.; Balderas, M.; Garcia-Pérez, E. *Tetrahedron Asymmetry* **1996**, *7*, 2233.

(5) We have no indication of racemization *en route* from the diastereoisomerically pure oxazolidinones to the β-amino acid and β-peptide derivatives described herein. The absolute configuration (*R*) of **3-5** follows from optical comparison.[6]

(6) Talley, J. J. (Monsanto Co., USA), Eur. Pat. Appl. EP 396 526, 1990; *Chem. Abstr.* **1991**, *114*, 229382.

(7) Houben-Weyl, *Synthese von Peptiden*, Volume 15, Part 1+2, Wünsch, E., Ed., G. Thieme, Stuttgart, 1974.

(8) This method has also been successfully employed in our synthesis of β^3-peptides.[1]

(9) We use the "old-fashioned" nomenclature for the absolute configuration in this case, because the (*R/S*) nomenclature gives opposite configurations for β-HVal *versus* β-HAla and β-HLeu! This is not the case for the β-amino acid residues in **9**.

(10) The insertion of helix-breaking elements (*e.g.* an amino acid of the "wrong" configuration, β,β-disubstituted amino acid, N-methyl-β-amino acid, 3-hydroxy-butyric acid or an α-amino acid) into β^3-peptides leads to a complete loss of the typical CD pattern.

(11) IR : *Perkin-Elmer 1600 FTIR*, $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AMX 500*, δ in ppm rel. to $SiMe_4$ (= 0 ppm), *J* in Hz; Carbon multiplicities were assigned by DEPT techniques. MS: *VG ZAB2-SEQ* with 3-nitrobenzyl alcohol (FAB, 3-NOBA).