210. Linear Stereo-co-oligopeptides with Alternating D- and L-Residues. I. Synthesis and Mass Spectrometric Behavior of the Members of a Series with Valine Residues

by **Lera Tomasic, Annibale Stefani** and **Gian Paolo Lorenzi**
Technisch-Chemisches Laboratorium, ETH-Zentrum, CH-8092 Zürich

(19.VIII.80)

Summary

The synthesis of the protected stereo-co-oligopeptides with alternating D- and L-valine residues Boc-(D-Val)$_m$-(L-Val-D-Val)$_n$-OMe (m=0; n=1,2,3,4,6,8, and m=1; n=1,2,3,4) is described. The synthesis has been carried out by using conventional solution methods. The co-oligopeptides up to the nonapeptide (m=1; n=4) have been prepared in a stepwise fashion. The dodecapeptide (m=0; n=6) and the hexadecapeptide (m=0; n=8) have been prepared by condensation of smaller peptides, and the criteria used for assessing their stereochemical purity are discussed. Preliminary characterization data are presented. Even the co-oligopeptides with six or more residues, up to the dodecapeptide included, have given EI/MS. showing the molecular ion peak. The possible reasons for this uncommon behavior are examined.

Introduction. – There have been several reports [1–6] in recent years indicating that co-polypeptide chains formed by the regular alternation of D- and L-residues may assume various types of regular conformations that are different from those known for poly-L-peptide chains. Of these specific conformations the β-helices are particularly interesting, since they appear to be involved in the formation of trans-membrane channels by the natural pentadecapeptide Gramicidin A [7]. The experimental characterization of these conformations, however, is in general poor. Also, the different factors that may contribute to the stabilization or destabilization of these conformations are not well known. With the aim of obtaining a deeper insight into the conformational characteristics of chains of alternating D- and L-aminoacid residues, we have initiated a systematic investigation of different series of alternating co-oligo-D,L-peptides differing in chain length and having identical side chains. Such co-oligopeptides necessarily consist of either enantiomeric or diastereomeric aminoacid residues, and for this reason we designate them as D,L-alternating (or syndiotactic) stereo-co-oligopeptides. Studies on series of such D,L-alternating stereo-co-oligopeptides seem to present special advantages for assessing the influence of the peptide chain length and of the nature of the lateral substituents on conformational properties. Indeed, in the case of L-peptides, similar conformational studies [8] on several series of homo-oligomers have proven extremely fruitful in this respect.

0018-019X/80/7/2000-05$01.00/0

Some preliminary results of our work have already been communicated [9–11]. In this paper we describe the synthesis of the D,L-alternating, protected stereo-co-oligopeptides II–IX, XII and XVI

$$\text{Boc-(D-Val)}_m\text{-(L-Val-D-Val)}_n\text{-OMe}$$

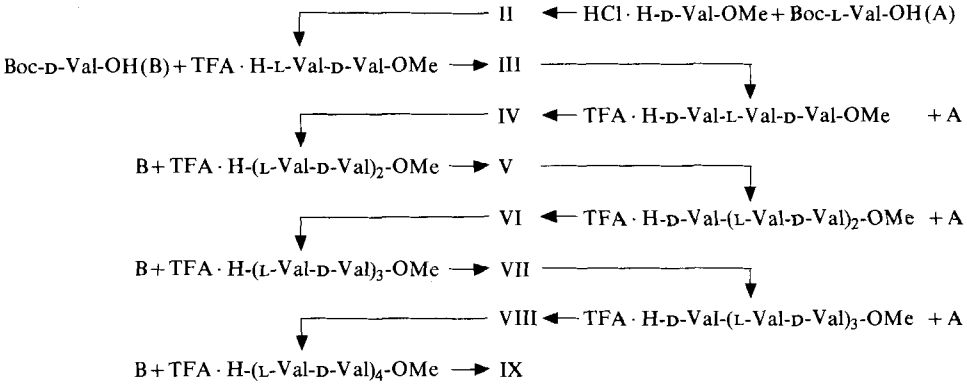
m = 0; n = 1,2,3,4,6,8 (II, IV, VI, VIII, XII, XVI)
m = 1; n = 1,2,3,4 (III, V, VII, IX)

of a series derived from D- and L-valine, and having a D-Val as the C-terminal aminoacid residue. The hexadecapeptide XVI contains exactly the same number of CONH-groups as Gramicidin A, and therefore its synthesis was considered useful for a future comparison of conformational properties with this antibiotic. The results of the mass spectrometric characterization of these co-oligopeptides seem to reflect interesting conformational characteristics in the crystalline state, and these results are discussed.

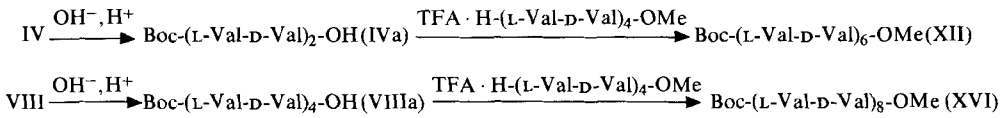
Synthesis. – The synthesis of the stereo-co-oligopeptides was carried out by using conventional solution methods. The oligomers from the tri- to the nonapeptide (III–IX) were prepared by stepwise elongation of the peptide chain starting from II *(Scheme 1)*. The dodecapeptide (XII) and the hexadecapeptide (XVI) were prepared by condensation of smaller peptide fragments *(Scheme 2)*. Each coupling reaction was performed by using the mixed-anhydride method, with isobutyl chloroformate as the mixed-anhydride-forming reagent [12]. The trifluoroacetic acid salts used for the coupling reactions were prepared by treatment with trifluoroacetic acid of the corresponding Boc-protected compounds. After purification, all oligomers showed only one spot by thin layer chromatography, and gave consistent mass spectra and correct elementary analyses *(Table)*.

In view of the strategy used for synthesizing XII and XVI, the possibility existed that these two products might be stereochemically impure, XII for the presence

Scheme 1. *Stepwise synthesis of the co-oligopeptides II–IX* (TFA, trifluoroacetic acid).



Scheme 2. *Synthesis of the co-oligopeptides XII and XVI* (TFA, trifluoroacetic acid).



especially of Boc-(L-Val-D-Val)-(L-Val-L-Val)-(L-Val-D-Val)$_4$-OMe, and XVI for that of Boc-(L-Val-D-Val)$_3$-(L-Val-L-Val)-(L-Val-D-Val)$_4$-OMe. This possibility of stereochemical contamination was checked by submitting the dodeca- and the hexadecapeptide to complete acid hydrolysis with 6N HCl at 110°, and measuring the optical activity of the hydrolysates. No loss of optical activity was found for a sample of L-valine, when it was treated similarly. The hydrolysates did not show any measurable optical activity even at the wavelength (225 nm) of the ORD. maximum of L-valine ($[\alpha]_{225}^{25} = +2560$). Under the conditions used, an excess of L- over D-valine of about 1% would have been detected. Thus, XII and XVI should contain, if any, less than 5% of an expected stereochemical impurity.

Mass spectrometric characterization. – As indicated in the *Table* even the co-oligopeptides VI–IX and XII gave mass spectra showing the molecular ion peak. This peak was rather intense, and corresponded to still more than 1% of the base peak at *m/z* 72 for the largest co-oligopeptides. Though not giving the molecular ion peak, the hexadecapeptide gave a fragment ion peak at *m/z* as high as 1387 (corresponding to a loss of H-Val$_3$-OMe from the molecular ion) *(Table)*.

These results must be viewed as unusual, considering that the mass spectra were obtained by using a normal EI. technique. In the case of linear L-peptides, even relatively short oligomers are usually not volatile enough, for the molecular ion peak

Table. *Some characterization data concerning the co-oligopeptides of the series Boc-(D-Val)$_m$-(L-Val-D-Val)$_n$-OMe with m=0, n=1,2,3,4,6,8* (II, IV, VI, VIII, XII, XVI) *and with m=1, n=1,2,3,4* (III, V, VII, IX).

Oligo-peptide	Recrystallization solvents	Analysis[a]) % C	% H	% N	Mol. weight (calc.)	Mass spectrum (M^+)	M.p. (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)[b])
II	Et_2O/Petroleum ether	58.16	9.15	8.48	330.43	330	110.7	−23.7 (1.02)
		58.16	9.06	8.42				
III	MeOH/H_2O	58.72	9.15	9.78	429.56	429	156.7	− 8.5 (1.00)
		58.74	9.14	9.66				
IV	MeOH/H_2O	59.07	9.15	10.60	528.69	528	209.6	−17.8 (1.00)
		59.07	9.16	10.74				
V	MeOH/H_2O	59.31	9.15	11.15	627.83	627	224.5	− 7.0 (1.10)
		59.27	9.26	11.04				
VI	MeOH/H_2O	59.48	9.15	11.56	726.96	726	232.7	−21.0 (1.02)
		59.39	9.16	11.38				
VII	MeOH/H_2O	59.61	9.15	11.87	826.09	825	235.3	−57.8 (1.02)
		59.54	9.02	11.70				
VIII	MeOH/H_2O	59.72	9.15	12.11	925.23	924	261.1	− 8.9 (1.00)
		59.56	9.22	12.07				
IX	EtOAc	59.79	9.15	12.31	1024.36	1023	[c])	−61.2 (1.16)
		59.14	8.99	12.11				
XII	$CHCl_3$/EtOAc	59.98	9.15	12.72	1321.76	1320	[c])	− 6.2 (1.12)
		59.41	8.99	12.21				
XVI	[d])	60.12	9.15	13.04	1718.30	1387[e])	[c])	−11.4 (1.05)
		59.26	9.14	12.82				

[a]) First line: Calc. second line: Found; [b]) The values in parentheses are the concentrations in g/100 ml; [c]) Decomposes before melting; [d]) The crude product was purified by exhaustive washing with MeOH; [e]) *m/z* of the peak of highest mass number in mass spectrum.

to be detected by this technique [13]. We were able to see molecular ion peaks only up to the pentapeptide by using comparable conditions with the Boc- and MeO-protected homo-oligopeptides of the series Boc-(L-Val)$_n$-OMe (n = 2–7), and the molecular ion peak shown by the pentapeptide was much less intense than that of V. Intermolecular H-bonds are responsible for this general behavior, and, in the case of the homo-oligo-L-valines, it is these bonds that strongly hold the peptide chains in a pleated sheet β-structure [14].

The relatively high volatility of the D,L-alternating stereo-co-oligopeptides with five or more valine residues, indicated by the mass spectrometric behavior and by other results (VIII could even be sublimated at 240° in high vacuum!) apparently calls for alternative crystal structures, where H-bonds do not contribute markedly to the lattice forces. Indeed, this is the situation in the case of crystalline VIII, the only member of the series whose crystal structure has been determined so far [11]. In the crystals VIII forms double β-helices with a characteristic nonpolar outer layer that is constituted by the isopropyl side chains of the valine residues. Thus, the lattice forces are mainly weak *van der Waals* interactions. These forces can be easily overcome and, under the conditions of the mass spectrometric determination, dissociation of the β-helical dimers to monomeric molecules may rapidly ensue. By analogy it seems quite possible that also the other D,L-alternating co-oligopeptides with five or more valine residues have crystal structures of the kind shown by VIII. However, other structural possibilities, in particular crystal structures with the peptide molecules in single-stranded β-helical conformations, are conceivable.

Conformational studies on these stereo-co-oligopeptides derived from valine are being carried out, and the results will be presented in future papers.

Experimental part. – *Materials and Methods.* D- and L-valine were products of *Fluka AG*, Buchs (Switzerland). Their optical rotations, $[\alpha]_D^{25} = -26.9$ for D- and $+27.0$ for L-valine ($c = 5.2$, 5N HCl), were measured and found to be in good accord with the literature values [15]. The samples of the series Boc-(L-Val)$_n$-OMe (n = 2–7) were a gift of Prof. *C. Toniolo* of the University of Padova, Italy. Thin-layer chromatography was performed on silica gel plates F_{254} of *Merck AG*, Darmstadt (BRD), by using a mixture $CHCl_3/CH_3OH/CH_3COOH$ 85:10:5 (*v/v*) as the eluent. Compounds were located with a ninhydrin spray and with iodine vapor. The microanalyses were performed by the microanalytical laboratory of the organic chemistry institute of the ETH, Zürich. The melting points were determined by differential thermal analysis using a *Mettler* TA 2000 System. Optical rotations at the sodium D-line were measured with a digital *Perkin-Elmer* model 141 polarimeter in a 100 mm thermostated cell. The ORD. measurements on the oligopeptide hydrolysates were performed with a *Cary* model 60 spectropolarimeter using 10 mm cells. The EI./spectra were measured using a *Hitachi* RMU-6L mass spectrometer for the co-oligopeptides II–IX (inlet temperature, 80–120°) and a *Varian* 711 instrument for XII and XVI (inlet temperature, 210° and 410° respectively). In all cases a ionizing energy of 70 eV was used.

Syntheses. – *Boc-D-Val-OH* was prepared using *Schnabel's* method [16], and was purified by recrystallization from ether/petroleum ether, m.p. 79.2°, $[\alpha]_D^{25} = -6.4$ ($c = 1.05$, acetic acid) (lit. [17]: m.p. 75.5–76.5°; $[\alpha]_D^{20} = -6.5$ ($c = 1.4$, acetic acid).

Boc-L-Val-OH was prepared and purified analogously, m.p. 79.5°; $[\alpha]_D^{25} = -6.5$ ($c = 1.02$, acetic acid) (lit.: m.p. 77–79° [18]; m.p. 72–73° [16]; $[\alpha]_D^{25} = -5.8$ ($c = 1.208$, acetic acid) [18]).

HCl · H-D-Val-OMe was prepared using the procedure employed by *Boissonnas et al.* [19] for the L-isomer, and was purified by recrystallization from methanol/ether, m.p. 166–168°; $[\alpha]_D^{21} = -16.2$ ($c = 1.99$, water) (lit. [20]: m.p. 167.5–168°; $[\alpha]_D^{21} = -15.6°$ ($c = 2$, water)).

II–IX, XII and *XVI* were prepared according to *Schemes 1* and *2*. The coupling reactions were carried out using the conditions and procedure described in a previous paper [21]. The work-up was made by washing the reaction mixture in chloroform with water, 1N HCl, 1N $NaHCO_3$ and again with water until the washings were neutral. After drying over anhydrous Na_2SO_4, the chloroform solution was

evaporated to dryness, and the crude product so obtained was generally purified by repeated recrystallizations until one spot only was observed by thin layer chromatography. The solvents used for recrystallizing the co-oligopeptides are indicated in the *Table*. The crude hexadecapeptide was purified by exhaustive washing with methanol.

Boc-(L-Val-D-Val)$_2$-OH (IVa) was prepared by alkaline hydrolysis of IV. To a solution of IV (1.09 g, 2.05 mmol) in 22 ml of methanol, 2.05 ml of 1N NaOH were added. The solution was stirred at room temperature for 24 h, and then 2.05 ml more of the base were added. The solution was kept stirring at room temperature for an additional 24 h, then the solvents were evaporated. Water (30 ml) was added to the residue, and the aqueous solution was washed with ethyl acetate (3 × 30 ml). Finally, 8.2 ml of 0.5N HCl were added. The precipitated product was collected by filtration, washed with water, and recrystallized from ethyl acetate/hexane, m.p. 162.3°; $[\alpha]_D^{25} = -5.4$ ($c = 1.25$, $CHCl_3$) (yield, 50%).

$C_{17}H_{30}N_4O_7$ Calc. C 58.34 H 9.01 N 10.89% Found C 58.77 H 9.25 N 10.23%

Boc-(L-Val-D-Val)$_4$-OH (VIIIa) was prepared in an analogous manner by hydrolysis of VIII. After washing with water the product was chromatographically pure, and was not recrystallized.

$C_{29}H_{50}N_8O_{11}$	Calc.	C 59.32	H 9.07	N 12.30%	C/N 4.82
	Found	„ 58.47	„ 9.02	„ 12.09%	„ 4.83

The authors wish to express their appreciation to Dr. *A. Lorenzi-Riatsch* of the University of Zürich, for performing the mass spectrometric determination with the *Varian* 711 spectrometer, and to *B. Straub* for performing the ORD. measurements and for reading the manuscript. The authors also thank Prof. *C. Toniolo* of the University of Padova, Italy, for the generous gift of the samples of homo-oligopeptides derived from L-valine.

This work has been supported by research grants 2.884-0.77 and 2.414-0.79 from the *Swiss National Science Foundation.*

REFERENCES

[1] *G.N. Ramachandran & R. Chandrasekaran,* Ind. J. Biochem. Biophys. *9,* 1 (1972).
[2] *F.T. Hesselink & H.A. Scheraga,* Macromolecules *5,* 455 (1972).
[3] *P. De Santis, S. Morosetti & R. Rizzo,* Macromolecules *7,* 52 (1974).
[4] *F. Heitz, B. Lotz & G. Spach,* J. Mol. Biol. *92,* 1 (1975).
[5] *F. Colonna-Cesari, S. Premilat, F. Heitz, G. Spach & B. Lotz,* Macromolecules *10,* 1284 (1977).
[6] *B.V. Venkatram Prasad & R. Chandrasekaran,* Int. J. Pept. Protein Res. *10,* 129 (1977).
[7] *S. Weinstein, B.A. Wallace, E.R. Blout, J.S. Morrow & W. Veatch,* Proc. Natl. Acad. Sci. USA *76,* 4230 (1979).
[8] *R.T. Ingwall & M. Goodman,* in: 'International Review of Science', Organic Chemistry Series Two, Vol. 6, H.N. Rydon, Ed., Butterworths, London, 153, 1976.
[9] *G.P. Lorenzi & T. Paganetti,* J. Am. Chem. Soc. *99,* 1282 (1977).
[10] *G.P. Lorenzi & L. Tomasic,* J. Am. Chem. Soc. *99,* 8322 (1977).
[11] *E. Benedetti, B. Di Blasio, C. Pedone, G.P. Lorenzi, L. Tomasic & V. Gramlich,* Nature *282,* 630 (1979).
[12] *J.R. Vaughan, jr. & R.L. Osato,* J. Am. Chem. Soc. *74,* 676 (1952).
[13] *E. Jäger,* in: «Methoden der Organischen Chemie» (Houben-Weyl), Vol. 15/2, E. Wünsch Ed., G. Thieme Verlag, Stuttgart, 716, 1974.
[14] *A. Del Pra & C. Toniolo,* Macromolecules *11,* 793 (1978).
[15] *S.M. Birnbaum, L. Levintow, R.B. Kingsley & J.P. Greenstein,* J. Biol. Chem. *194,* 455 (1952).
[16] *E. Schnabel,* Liebigs Ann. Chem. *702,* 188 (1967).
[17] *B.F. Gisin, R.B. Merrifield & D.C. Tosteson,* J. Am. Chem. Soc. *91,* 2691 (1969).
[18] *G.W. Anderson & A.C. McGregor,* J. Am. Chem. Soc. *79,* 6180 (1957).
[19] *R.A. Boissonnas, St. Guttmann, R.L. Huguenin, P.-A. Jaquenoud & Ed. Sandrin,* Helv. *41,* 1867 (1958).
[20] *E.L. Smith, D.H. Spackman & W.J. Polglase,* J. Biol. Chem. *199,* 801 (1952).
[21] *U. Widmer, G.P. Lorenzi & P. Pino,* Biopolymers *18,* 239 (1979).