94. Some Cyclic Oligopeptides with S_{2n} Symmetry

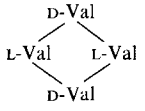
by **Lera Tomasic** and **Gian Paolo Lorenzi***

Institut für Polymere, ETH-Zentrum, CH-8092 Zürich

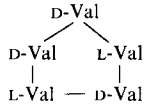
(30.III.87)

Some cyclic oligopeptides formed by an equal number of alternating D- and L-amino-acid residues have been synthesized by using the hydrochloride of the open-chain peptide acid as precursor and the mixed-anhydride condensation method. The cyclic oligopeptides (tetra-, hexa-, and octavaline, hexaleucine, and hexaphenylalanine) form very stable H-bonded structures (IR-amide band at 3270–3290 cm^{-1}) which are insoluble in common organic solvents. In $CF_3COOH/CDCl_3$ (25°), they yield ^1H-NMR spectra showing the expected equivalency of the various amino-acid residues.

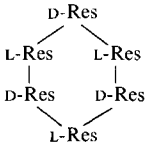
Introduction. – Cyclic oligopeptides formed by an equal number (n) of alternating D- and L-amino-acid residues can assume, in principle, a β-type conformation and in this conformation stack to form tubular structures stabilized by parallel or antiparallel H-bonds [1]. These structures bear strong resemblance to β-helices and, as some β-helices do [2], may be able to establish conducting pores across suitable films. We intend to investigate this possibility by using cyclic oligopeptides consisting of residues which are enantiomeric (stereocooligopeptides [3]). Cyclopeptides of this kind have been noted [4] for their peculiar nature of *meso* compounds with an S_{2n} symmetry axis, but so far only the cyclo(hexaphenylalanine) and the cyclo(octaphenylalanine) have received some experimental attention [5]. Here we report on the synthesis of the cyclic oligovalines **1**, **3**, and **4** with $n = 2$, 3, and 4, respectively, of cyclo(hexaleucine) **5** and of cyclo(hexaphenylalanine) **6**. The related but asymmetric cyclo(pentavaline) **2** is included for the sake of comparison.



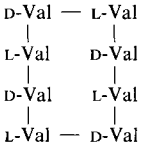
1



2



3 Res = Val
5 Res = Leu
6 Res = Phe



4

Synthesis. – The cyclic oligomers **1**–**6** have been synthesized from the hydrochlorides of the corresponding open-chain peptide acids by using the mixed-anhydride method as indicated in the *Scheme*. This method has been used for peptide cyclization only seldom [6–8], but it has appealed to us because of its simplicity. The percent conversions of the precursor into the desired cyclic oligomer is given in *Table 1*. The limitedness at least of some of these conversions must be attributed not only to concurrent intermolecular

Scheme. *Synthetic Approach Used for the Cyclic Oligomers* **1** *and* **3–6**. The approach used for **2** was similar. ○ = L-Val, D-Leu or D-Phe; ● = corresponding enantiomeric residue. MeMo = *N*-methylmorpholine.

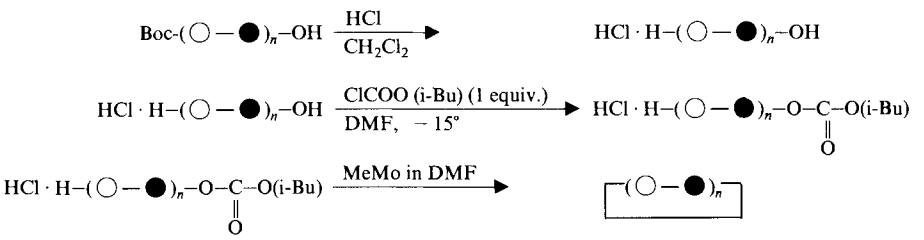


Table 1. *Extent of Condensation and Conversion in the Various Cyclization Reactions*[a])

Starting precursor		Overall condensation[b]) [%]	Conversion of precursor into desired oligomer[c]) [%]	Other cyclic products formed
HCl · H–(L-Val-D-Val)$_2$–OH	1st run	Not determined	1 (**1**)	Octamer **4**[d])
	2nd run	75	3 (**1**)	Octamer **4**[e])
HCl · H–(L-Val-D-Val)$_3$–OH	1st run	40	16 (**3**)	Not recognized
	2nd run	Not determined	19 (**3**)	Not recognized
HCl · H–(L-Val-D-Val)$_4$–OH		29	4 (**4**)	Epimerized octamer[f])
HCl · H–(D-Leu-L-Leu)$_3$–OH		40	13 (**5**)	Not recognized
HCl · H–(D-Phe-L-Phe)$_3$–OH		Not determined	17 (**6**)	Not recognized
HCl · H–D-Val-(L-Val-D-Val)$_2$–OH		Not determined	28 (**2**)	Not recognized

[a]) The conditions (see *Exper. Part*) were the same in all experiments, except in that with hexaleucine.

[b]) Percentage of intra- and intermolecularly reacted amino groups. Calculated from the ^{1}H-NMR spectrum (CF_3COOH/CD_2Cl_2 2:1) of the crude product as $[(1 - RM)/(1 + R)] \cdot 100$, R being 1/3 of the ratio of the intensity of the NH_3^+ signal to the total intensity of the NH signals and M the number of NH in the starting peptide. The crude product was obtained by evaporating the solvent from an aliquot portion of the reaction mixture and washing the residue with H_2O exhaustively.

[c]) Based on the amount of the cyclic oligomer in the DMF-insoluble material formed in the reaction, as determined by ^{1}H-NMR analysis.

[d]) Amount comparable to that of **1**.

[e]) Traces.

[f]) About half the amount of **4**.

condensation, but also to incomplete mixed-anhydride formation (evidenced in *Table 1* by the low extents of overall condensation). This is possibly the consequence of poor reactivity of the carboxyl groups [7], but other reasons (impurities in the solvent used) cannot be ruled out. The data of *Table 1* indicate that the pentavaline and the hexapeptides undergo cyclization more easily than the tetra- and octavaline.

Characterization. – The cyclic oligomers synthesized are insoluble in all common organic solvents. Cyclopeptides **1**, **2**, **4**, and **5** can be dissolved in CF_3COOH, but **3** and **6** are insoluble even in CF_3COOH. *Nishino et al.* have reported [5] the purification of cyclo(L-Phe-D-Phe)$_3$ (**6**) by silica-gel chromatography using $CHCl_3$/MeOH 98:2. We have tried this solvent mixture with **6** without success. Solutions of **6** as well as of **3** could be obtained only by using mixtures of CF_3COOH and $CHCl_3$ or CH_2Cl_2.

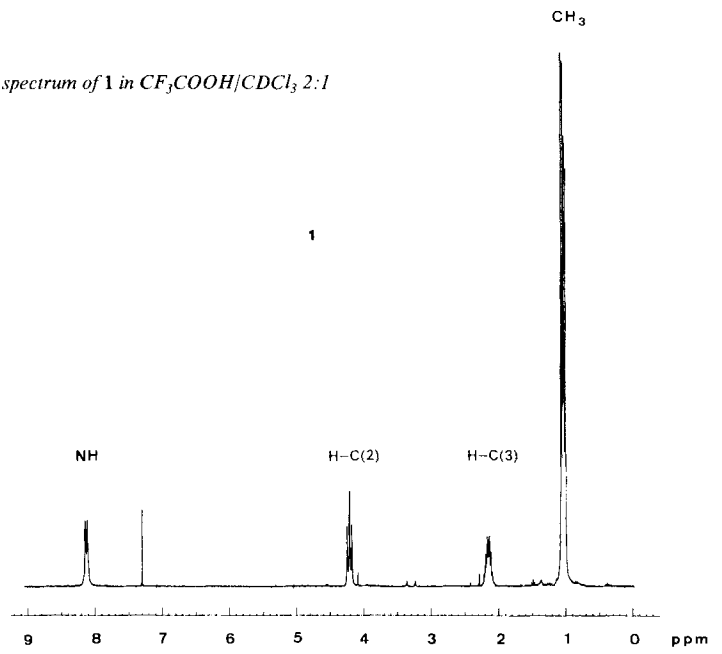
Table 2. *^{1}H-NMR Data for the Cyclic Oligomers* **1-6** *in $CF_3COOH/CDCl_3$ 2:1 at 25°* [a])

Oligomer	NH		H–C(2)		H–C(3)	H–C(4)	H–C(5)
	δ[b])	J(HN,H–C(2))[c])	δ[b])	J(H–C(2),H–C(3))[c])	δ[b])	δ[b])	δ[b])
1	8.12	9.6	4.21	10.4	2.17	1,06, 1.03	–
3	8.17	8.3	4.34	8.4	2.13	1.08, 1.07	–
4	8.18	9.2	4.84	9.1	2.20	1.09, 1.03	–
5	8.15	8.2	4.55		1.65	1.65	1.00, 0.95
6	7.79	8.4	4.67		2.72	–	7.27 or 6.89[e])
2[d])	8.41	9.6	4.33	9.6			
	8.24	9.0	4.23	9.6			
	8.03	7.4	4.23	9.6	2.2–1.8	1.1–0.9	–
	7.57	9.3	4.13	9.2			
	7.10	7.4	3.87	10.8			

[a]) Concentrations in the range 2–8 mg/ml.
[b]) In ppm rel. to TMS (= 0 ppm); ± 0.03 ppm.
[c]) In Hz; ± 0.3 Hz.
[d]) Data for NH and H–C(2) on the same line do not necessarily refer to protons of the same residue.
[e]) In the case of **6**, H–C(5) means the 2 H_o of Ph. The signal at 7.27 ppm represents 3 arom. H, one of them being H_p.

In the ^{1}H-NMR spectrum ($CF_3COOH/CDCl_3$ 2:1), **2** shows different signals for protons of the same kind in different residues *(Table 2)*. By contrast, all other oligomers give a unique signal for protons of the same kind, thus showing that the residues are equivalent, as expected. Note *(Table 2)* that oligomers differing in ring size give signals at

Fig. 1. *^{1}H-NMR spectrum of* **1** *in $CF_3COOH/CDCl_3$ 2:1*



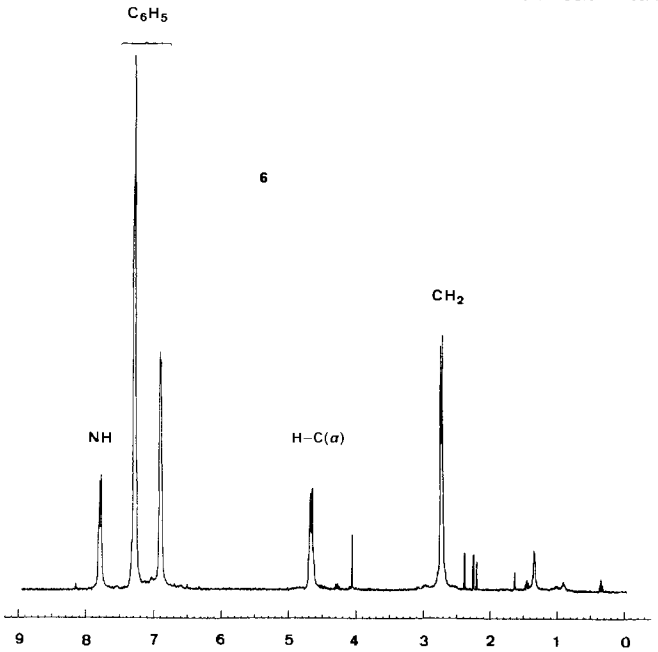


Fig. 2. *^{1}H-NMR spectrum of* **6** *in $CF_3COOH/CDCl_3$ 2:1.* The signal near 4 ppm derives from an impurity in CF_3COOH. The signals below 2.5 ppm derive from impurities which have not been identified.

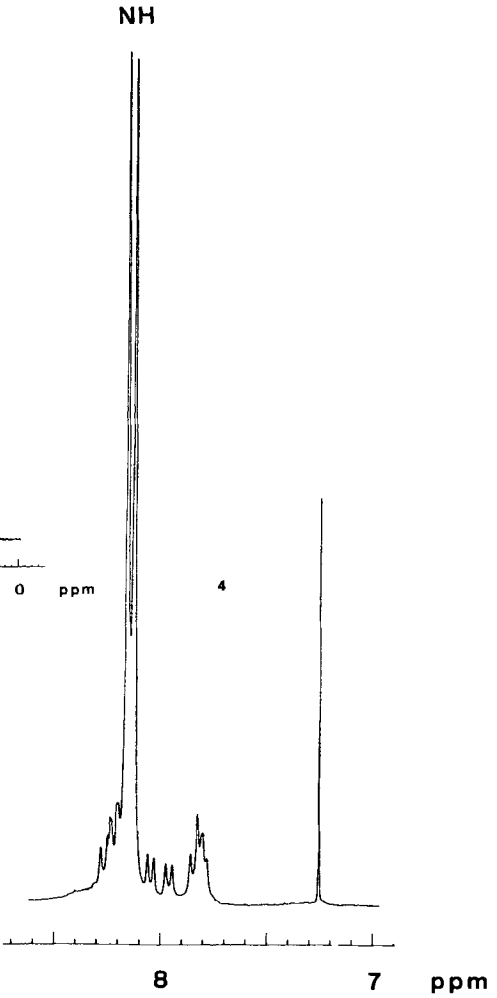


Fig. 3. *NH Region of the ^{1}H-NMR spectrum of* **4** *in $CF_3COOH/CDCl_3$ 2:1.* The *d* of the main product is accompanied by the signals of an impurity.

different spectral positions. They can thus be recognized even when occurring together (*Table 1*, last column). ^{1}H-NMR spectra of purified samples of **1** and **6** are shown in *Figs. 1* and *2*, respectively, to provide an indication of the degree of purity attained. Spectra of comparable quality have been obtained from purified samples of **3** and **5**. The final spectrum of **4** *(Fig. 3)* indicates the presence of a rather large amount of a peptidic impurity that we have not been able to remove. This impurity – a cyclic one, since the characteristic, broad NH_3^+ signal at *ca.* 7.25 ppm of the open-chain oligomers is absent – is very likely an epimer of **4** resulting from some racemization during the cyclization.

In CF_3COOH or in solvent mixtures such as those used for the NMR spectra, H-bonds between peptide molecules are not stable. Therefore, under these solution conditions, **1** and **3**–**6** cannot be expected to form tubular aggregates of the type foreseen [1]. It is possible that these cyclic peptides may exhibit ring stacking with interannular

H-bonds in the solid state. This possibility which is consistent with IR results (amide-A bands at 3270–3290 cm^{-1}) indicating the presence of strong H-bonds is currently being investigated by X-ray diffraction of single crystals.

The authors thank *F. Bangerter* for the NMR measurements.

Experimental Part

General. Solvent mixtures in *v/v* ratios. M.p.: *Kofler* hot stage microscope. IR spectra (KBr): *Perkin Elmer 177* grating spectrophotometer. ^{1}H-NMR spectra (25°): *Bruker AM-300* spectrometer. MS: *Hitachi Perkin Elmer* model *RMU-6L* spectrometer.

Boc-Protected Oligopeptide Acids. Boc-(L-Val-D-Val)$_2$-OH and Boc-(L-Val-D-Val)$_4$-OH were prepared and purified as already described [3]. The other compounds were prepared analogously by hydrolysis of the corresponding methyl esters Boc-D-Val-(L-Val-D-Val)$_2$-OMe [3], Boc-(L-Val-D-Val)$_3$-OMe [3], Boc-(D-Leu-L-Leu)$_3$-OMe [9], and Boc-(D-Phe-L-Phe)$_3$-OMe [10]. After purification, all oligopeptides gave a single spot on TLC (silica gel, $CHCl_3$/MeOH/AcOH 85:10:5) and ^{1}H-NMR ($CDCl_3$) in full accord with the structures expected.

Hydrochlorides. General Procedure. Dry HCl was passed at r.t. over a stirred soln. of the Boc-oligopeptide acid in CH_2Cl_2 or (case of Boc-D-Val-(L-Val-D-Val)$_2$—OH) dioxane. The completion of the reaction was ascertained by TLC. The insoluble hydrochloride was isolated by evaporation of the solvent and dried at 60° *in vacuo* overnight. The ^{1}H-NMR ($CF_3COOH/CDCl_3$ 2:1) did not reveal impurities, but the final weight was sometimes greater than expected, indicating the presence of more than 1 equiv. of HCl. The excess HCl was taken into consideration in calculating the amounts of reagents to be used in the cyclization step.

*Cyclizations to Cyclo(*L-*Val*-D-*Val*-L-*Val*-D-*Val)* (**1**), *Cyclo(*D-*Val*-L-*Val*-D-*Val*-L-*Val*-D-*Val)* (**2**), *Cyclo(*L-*Val*-D-*Val*-L-*Val*-D-*Val*-L-*Val*-D-*Val)* (**3**), *Cyclo(*L-*Val*-D-*Val*-L-*Val*-D-*Val*-L-*Val*-D-*Val*-L-*Val*-D-*Val)* (**4**), *Cyclo(*L-*Leu*-D-*Leu*-L-*Leu*-D-*Leu*-L-*Leu*-D-*Leu)* (**5**), *and Cyclo(*L-*Phe*-D-*Phe*-L-*Phe*-D-*Phe*-L-*Phe*-D-*Phe)* (**6**). *General Procedure.* The hydrochloride (*ca.* 500 mg) was dissolved in DMF (*Aldrich*, 'Gold Label'; *ca.* 1 mmol peptide/60 ml DMF). The soln. was cooled to −15° and 1 equiv. of isobutyl chloroformate in DMF (1 mmol/ml) was added under stirring. After 15 min, the dropwise addition of *N*-methylmorpholine in DMF (1 mmol/5 ml) was started. The addition was continued at a constant rate of *ca.* 5 ml/h until the amount of base added corresponded to twice the maximum theoretical amount of combined and free HCl. [In the case of the hexaleucine hydrochloride (1.31 mmol; excess HCl, 1 equiv.), a soln. in DMF with the same concentration as generally used could be obtained only by passing dry HCl over the heterogeneous mixture initially obtained. The final excess HCl, as determined by titration, was *ca.* 30 equiv. The *N*-methylmorpholine (62.3 mmol in 330 ml of DMF) was added dropwise within 6 h.] After completion of the addition, the cooling bath was removed and the mixture stirred overnight. The DMF-insoluble product formed was collected by filtration and washed on the filter with DMF (**1**, **2**, **4**, and **5**) or CF_3COOH (**3** and **6**). In the case of **1** ($M^{+\cdot}$ 396) and **2** ($M^{+\cdot}$ 495) final purification was achieved by recrystallization from CF_3COOH/MeOH, and in the case of **3** by recrystallization from $CF_3COOH/CHCl_3$/EtOAc. None of the cyclic oligopeptides melted in the investigated range (up to 250°).

REFERENCES

[1] P. De Santis, S. Morosetti, R. Rizzo, *Macromolecules* **1974,** *7,* 52.
[2] B.A. Wallace, *Biophys. J.* **1986,** *49,* 295, and ref. cit. therein.
[3] L. Tomasic, A. Stefani, G.P. Lorenzi, *Helv. Chim. Acta* **1980,** *63,* 2000.
[4] V. Prelog, H. Gerlach, *Helv. Chim. Acta* **1964,** *47,* 2288.
[5] N. Nishino, M. Ishiyama, T. Fukuda, T. Fujimoto, in 'Peptide Chemistry 1984', Ed. N. Izumiya, Protein Research Foundation, Osaka, 1985, pp. 371–374.
[6] R.A. Boissonnas, I. Schumann, *Helv. Chim. Acta* **1952,** *35,* 2229.
[7] T. Wieland, J. Faesel, H. Faulstich, *Liebigs Ann. Chem.* **1968,** *713,* 201.
[8] H. Faulstich, E. Nebelin, T. Wieland, *Liebigs Ann. Chem.* **1973,** 50.
[9] L. Tomasic, G.P. Lorenzi, in preparation.
[10] G.P. Lorenzi, C. Gerber, H. Jäckle, *Macromolecules* **1985,** *18,* 154.