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SYNTHESIS AND NMR INVESTIGATION OF CYCLIC PENTAPEPTIDE ANALOGUES OF THYMOPENTIN

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Summary: The synthesis of two cyclic pentapeptides cyclo(-Arg-Lys-Xxx-D-Val-Tyr-) (Xxx=Asp or Glu) with thymopentin-analogue sequences is described. Cyclization was achieved by the carbodiimide/DMAP method. The results of the NMR investigations performed on the protected pentapeptides suggest a βII'/γ-structure in DMSO solution.

Several peptides, especially thymosin and thymopoietin, which contribute to the differentiation ("maturation") of the thymus-dependent lymphocytes (T cells) have been isolated from thymus extracts. The active sequence of thymopoietin II, the pentapeptide Arg-Lys-Asp-Val-Tyr (TP-5, now called thymopentin)[1] exhibits thymopoietin-like action and the substance is therapeutically used in clinical tests as an immunostimulant[1b,2].
In order to develop more rigid analogues of the thymopentin we synthesized the cyclic pentapeptides cyclo(-Arg-Lys-Xxx-D-Val-Tyr-) (3:Xxx=Asp; 4:Xxx=Glu). For building up the peptide chain by conventional peptide synthesis (fragment condensation) we used PPA[3] as coupling reagent (Fig.1). The Boc and t-butyl ester protecting groups were removed by trifluoroacetic acid. Hydrogenolytically removable protecting groups were used for side-chain protection (deprotection: 10% Pd-charcoal in acetic acid/methanol). The crucial cyclization step was carried out in DMF-CH_2Cl_2 solution by carbodiimide (20 equiv. of DCC or EDCI) with addition of DMAP (5-10 equiv.) as an acylation catalyst already used in peptide synthesis[4]. Application of this method to a variety of different peptides[5] showed advantages of higher yields and higher purities[6a] compared to the azide method by Medzhiradsky. Nevertheless, as indicated in Fig. 1 the strong activation leads to isolated products with configuration inversion of the C-terminal amino acid, if the linear precursor contains no C- or N-terminal D-amino acid[5]. We conclude that the conformation of such a linear precursor is unfavorable for cyclization. The C-terminal inversion[6b] caused by the cyclization reagent now enables an easy reaction.

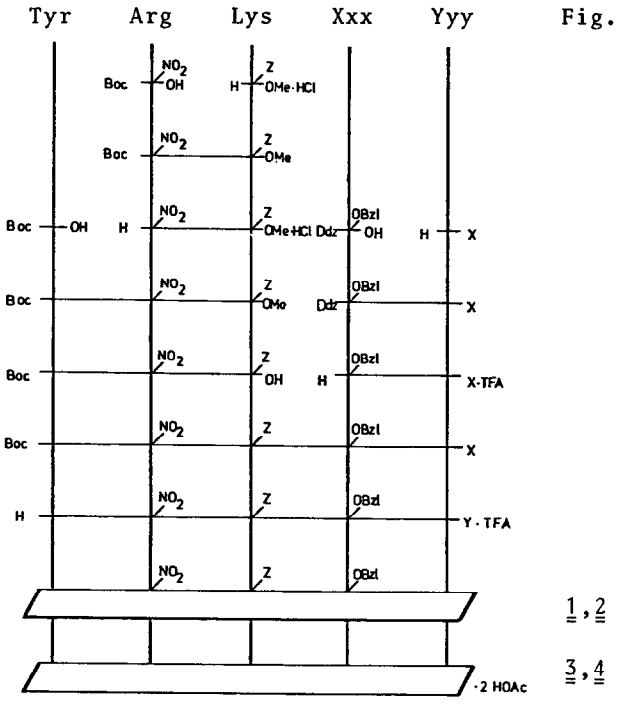

Fig.1. Synthesis of cyclic thymopentin analogues (X = OBu^t; Y = OH)

	Xxx	Yyy	cyclization yield[a]
1	Asp	L-Val	30% [b]
	Asp	D-Val	46%
2	Glu	L-Val	33% [b]
	Glu	D-Val	46%

a) All cyclic products contain Yyy = D-Val; b) C-terminal inversion during cyclization with EDCI/DMAP.

The ^1H, ^{13}C, and ^{15}N NMR spectra of the protected cyclopeptides 1 and 2 in DMSO were assigned via two-dimensional NMR spectroscopy (COSY[7]; NOESY[8]; H,C-COSY[9]; H,N-COSY[10]; H,C-COLOC[11])[5]. The conformationally relevant NMR data of 1 and 2 are summarized in Table I. The backbone conformation (Fig.2) is derived in the usual way[12] from the temperature coefficients of the amide-protons, HN-C_αH-coupling constants, NOE effects, and ^{15}N chemical shifts. The data are consistent with a βII'/γ-structure for 1 and 2 with internally oriented amide protons of Arg as well as Asp (1) and Glu (2), respectively. The βII'-turn involves the amino acids Glu-D-Val-Tyr-Arg, the γ-turn the residues Arg-Lys-Glu. The NH-temperature gradient of Lys is rather small. This can be explained by a folding back of the Lys-side chain. The carbonyl group of the Z group then might act as a hydrogen acceptor group for bridging. Similar results were obtained in other Lys peptides[13]. Further investigations about this are required. The exchange of Asp (1) vs. Glu (2) results in slightly different backbone properties as e.g. stated by the more differentiated temperature coefficients of the amide protons in the Glu-peptide 2[5]. Recent ^1H-NMR investigations of thymopentin[14] and its Glu^3 -analogue Arg-Lys-Glu-Val-Tyr (SP-5)[15] showed related results which parallel the different biological properties of both linear peptides.

Table I. NMR data of 1 and 2 in DMSO

		Arg	Lys	Asp/Glu	D-Val	Tyr
δ_{NH} [ppm]	1	7.62	8.30	8.08	8.32	8.41
	2	7.38	8.29	7.68	8.70	8.62
$\Delta\delta/\Delta T$ (NH) [10^{-3} ppm K^{-1}]	1	2.2	2.9	1.1	5.3	4.9
	2	0.7	1.9	-0.7	6.5	6.7
$^3J_{HNC_\alpha H}$ [Hz]	1	8.6	7.7	7.9	8.1	7.7
	2	9.9	7.0	8.6	7.1	8.5
δ_N [ppm CH_3NO_2 ext.]	2	-266.0	-262.5	-271.1	-258.5	-259.3
NOE effects[a] [in %]	1	R_α 5.6 Y_α 4.5 Y_N 3.6	K_α 8.8 R_α 6.6 D_N 2.7	D_α 6.3 K_α 5.1	v_α 3.5 v_β 4.2 D_α 8.4	Y_α 4.3 Y_β 3.4 v_α 15.6 R_N 4.9
	2	R_α 6.2 Y_α 4.5 Y_N 4	K_α 10.6 R_α 10.6 E_N 5	E_α 6.7 E_β 9.8 K_α 5.6 K_N 2.4	v_α 4.7 v_β 10.1 E_α 17.6	Y_α 6.9 Y_β 3.4 v_α 13.8 R_N 6.9

a) All NOE effects are negative. NH protons were irradiated. The amino acids are abbreviated by the IUPAC one letter symbols (R = Arg, K = Lys, D = Asp, E = Glu, v = D-Val, Y = Tyr). E.g. R_α means an NOE effect observed at the Arg-C_αH proton.

The deprotected peptides 3 and 4 show high activity in the phythämagglutinine and the plaque forming cell assay[16]. The chirality of the amino acids in the sequence of cyclic thymopentin analogues strongly determines the conformation of the backbone as well as the biological activity. 3 and 4 are the most active compounds among ten cyclic pentapeptide analogues which we have investigated so far[5]. Hence the conformation shown in Fig.2 should not be too much different from the biologically active conformation. Another cyclic thymopentin analogues of low biological activity[17] was reported recently[18].

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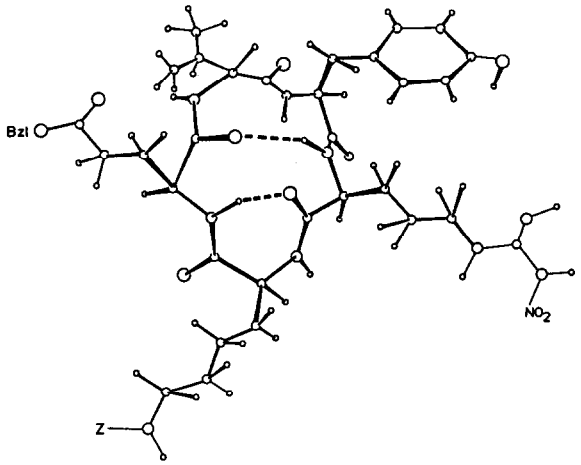
Fig.2. Conformation of 2 in DMSO solution



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