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PEPTIDE CONFORMATIONS, 23[1)]
NMR INVESTIGATIONS OF CYCLIC HEXAPEPTIDES CONTAINING THE ACTIVE SEQUENCE OF SOMATOSTATIN

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Summary: The conformation of five cyclic hexapeptides of the constitution cyclo[Phe-Phe-Trp-Lys-Thr-Phe], in which the chirality of Trp and two of the Phe residues is varied, has been investigated by NMR spectroscopy.

Earlier studies of cyclic enkephaline derivatives have shown that the conformation of cyclic peptides of a given constitution is strongly determined by the chirality of the amino acid residues[2)]. Hence variation of the chirality offers the possibility to change the conformation of an active sequence within such a cyclic peptide[3)]. We report here conformational studies of five of the eight possible cyclic hexapeptides of the constitution cyclo[Phe^6-Phe^7-Trp^8-Lys^9-Thr^{10}-Phe^{11}][4)] in which the configuration of the amino acids 6,8, and 11 was changed as follows (all others are L configurated):

1: cyclo[L-Phe-Phe-D-Trp-Lys-Thr-D-Phe]
2: cyclo[L-Phe-Phe-D-Trp-Lys-Thr-L-Phe]
3: cyclo[L-Phe-Phe-L-Trp-Lys-Thr-D-Phe]
4: cyclo[D-Phe-Phe-D-Trp-Lys-Thr-L-Phe]
5: cyclo[D-Phe-Phe-L-Trp-Lys-Thr-L-Phe]

The synthesis of the peptide 2 has already been described as part of the elegant work of VEBER et al. during the synthesis of biologically active cyclic mini-somatostatins [5)].

The ^{1}H-NMR spectra in DMSO were assigned via modern NMR techniques such as difference NOE measurements and 2D-spin-echo-correlated spectroscopy (SECSY)[6)].

The SECSY spectrum yields in a rapid and easily interpretable manner the connectivities within the spin systems of the amino acids. As an example the SECSY spectrum of 2 is shown in Fig. 1 (following page). By this way Thr and Lys are directly assigned and used as starting points for the sequencial assignments via the NOE effects between the NH-protons and the α-protons of the preceding amino acid in the sequence[3].

Of special importance for the conformational interpretation are the chemical shift values, their temperature coefficients, and the coupling constants of the NH-protons (Table I).

Table I. ^{1}H-NMR data of the NH-protons of cyclic hexapeptides in DMSO

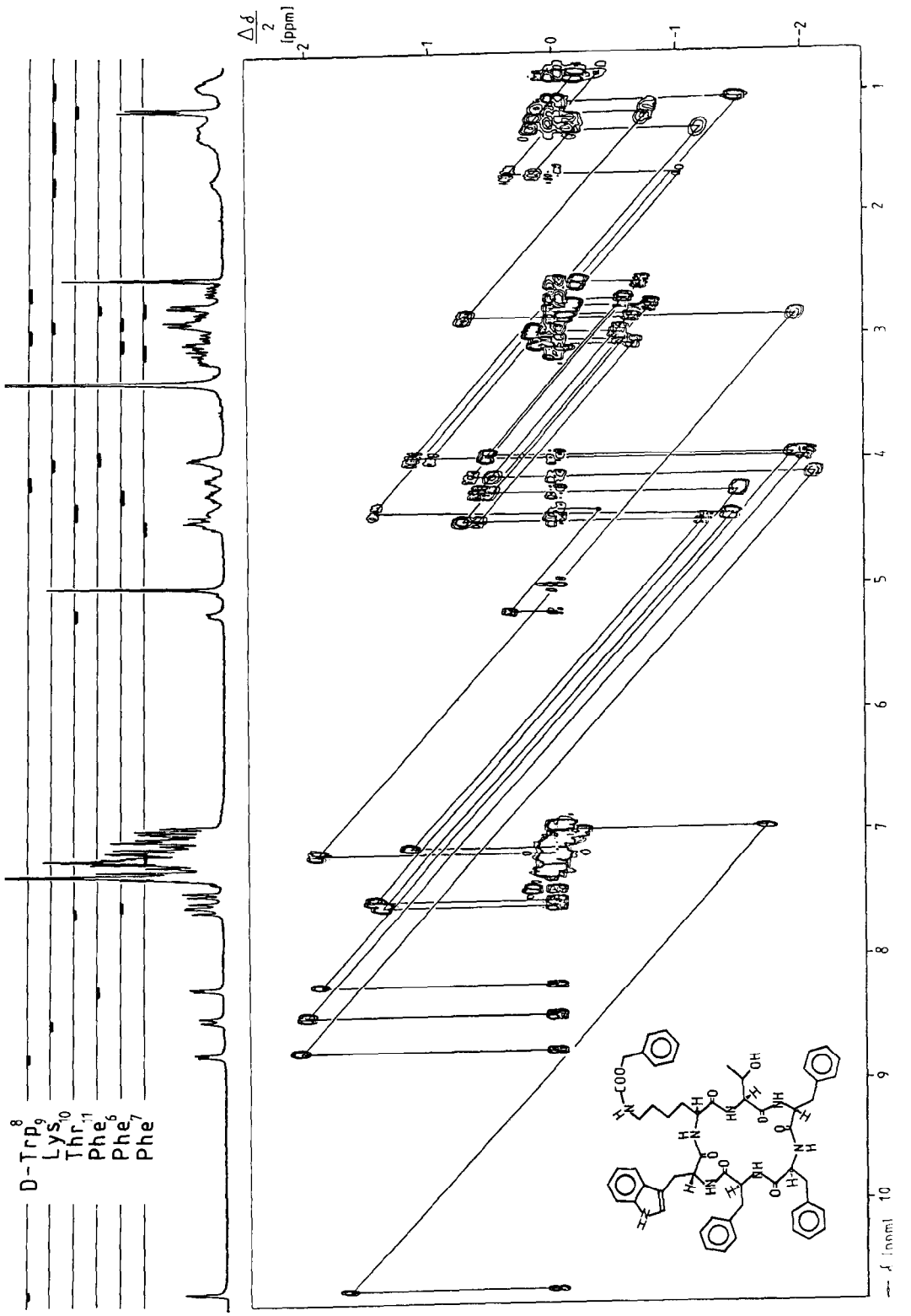
		Phe^6	Phe^7	Trp^8	Lys^9	Thr^{10}	Phe^{11}
δ [ppm]	1	8.54	7.20	8.58	8.70	7.09	8.14
	2	7.59	7.15	8.79	8.50	7.63	8.28
	3[b]	8.29	7.99	8.45	7.71	7.93	8.60
	4	8.60	7.60	8.36	8.46	7.49	7.77
	5	8.57	7.18	8.00	8.42	7.26	8.65
$\Delta\delta/\Delta T$ [$\cdot 10^{-3}$ppm K^{-1}]	1	9.1	-0.2	5.9	6.5	-2.4	6.1
	2	1.9	1.1	5.0	4.7	1.9	3.5
	3[b]	2.9	4.7	4.8	1.2	5.2	6.9
	4	5.0	1.3	4.2	4.6	1.3	3.0
	5	5.9	7.1	3.5	5.2	0.4	-0.1
$^3J_{HNC_\alpha H}$ [Hz]	1	8.5	6.5	5.9	7.2	8.1	7.0
	2	8.5	6.4	5.1	8.7	9.5	4.2
	3[b]	6.8	8.7	8.3	8.3	4.2	7.2
	4	9.4	6.4	6.1	8.5	8.3	4.8
	5	5.3	8.6	9.4	4.2	7.3	4.4

a) Temperature gradients in 10^{-3} ppm/K between 20 and 60° C.

b) The signals of the aromatic amino acids (Phe, Trp) are not assigned yet because of strong peak overlap.

Studies of cyclic hexapeptides so far often resulted in a conformation containing two β-turns[7]. Such a conformational proposal is also consistent with our results of the peptides 1 and 4: two small temperature coefficients for Phe^7 and

Figure 1. 270 MHz- SECSY spectrum of 2 in DMSO (next page).


D-Trp 8
Lys 9
Thr 10
Phe 11
Phe 6
Phe 7
Δδ/2 [ppm]
f [ppm]

Thr^{10} which are thus considered as internally oriented. The very large differences $\Delta\delta/K(NH)$ in 1[8], which has a symmetrical $(DLL)_2$-sequence of the amino acids, strongly point to a preferred conformation with βII' turns of the amino acids 7,8,9,10 and 10,11,6,7. In 4 the effect is less pronounced whereas the interpretation of the results of 2 on the basis of a two-β-turn-structure requires the additional assumption of a sterically shielded Phe^6-NH-proton (model considerations). Strong peak overlap in 3 does not enable us to assign all resonances but the large NH temperature coefficient of Thr^{10} excludes a similar conformation as of 1 and 4. In 5, in contrast of previous results on cyclic hexapeptides, obviously the NH protons of two adjacent amino acids are inwardly oriented with respect to the usual interpretation of their temperature coefficients. This fact requires further investigations.

All peptides do not exhibit any significant biological somatostatin activity.

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