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PEPTIDE CONFORMATIONS, 24[1)]
HOMO- AND HETERONUCLEAR 2D NMR SPECTROSCOPY OF CYCLIC PENTAPEPTIDES CONTAINING THE ACTIVE SEQUENCE OF SOMATOSTATIN

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Summary: 2D NMR methods such as SECSY and 2D-^{1}H-^{13}C-shift correlations enable assignments of the NMR signals in three cyclic peptides of the constitution cyclo|Phe-Trp-Lys-Thr-Pro|

In continuation of our previous work on cyclic pentapeptides[2)] and cyclic somatostatin derivatives[1)] we report here the NMR studies of three peptides in which the active sequence of somatostatin (Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10}) is bridged by a proline residue[3)]. The chirality of Trp^{8} and Thr^{10} was varied as follows:

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

The ^{1}H NMR spectra were analyzed via NOE difference spectroscopy and 2D spin echo correlated spectroscopy (SECSY) as described for the similar cyclic hexapeptides[1)]. The two dimensional techniques provide several advantages over classical methods. Especially the coupling of proline and lysine multiplets is clearly represented by cross-peaks in the SECSY-spectrum but nearly impossible to elucidate by difference decoupling.

The presence of proline in the sequence requires the investigation of cis-trans-isomerism of the Thr-Pro bond which is best done via C-13 NMR spectroscopy[2)]. On the other hand, the simultaneous presence of Lys results in more signals in the relevant region between 20 and 35 ppm. An unambiguous assignment of the proline carbon signals (as well as the others) therefore has been performed by 2D-^{1}H-^{13}C-shift correlations[5)]. As an example the spectrum of 1 is shown in

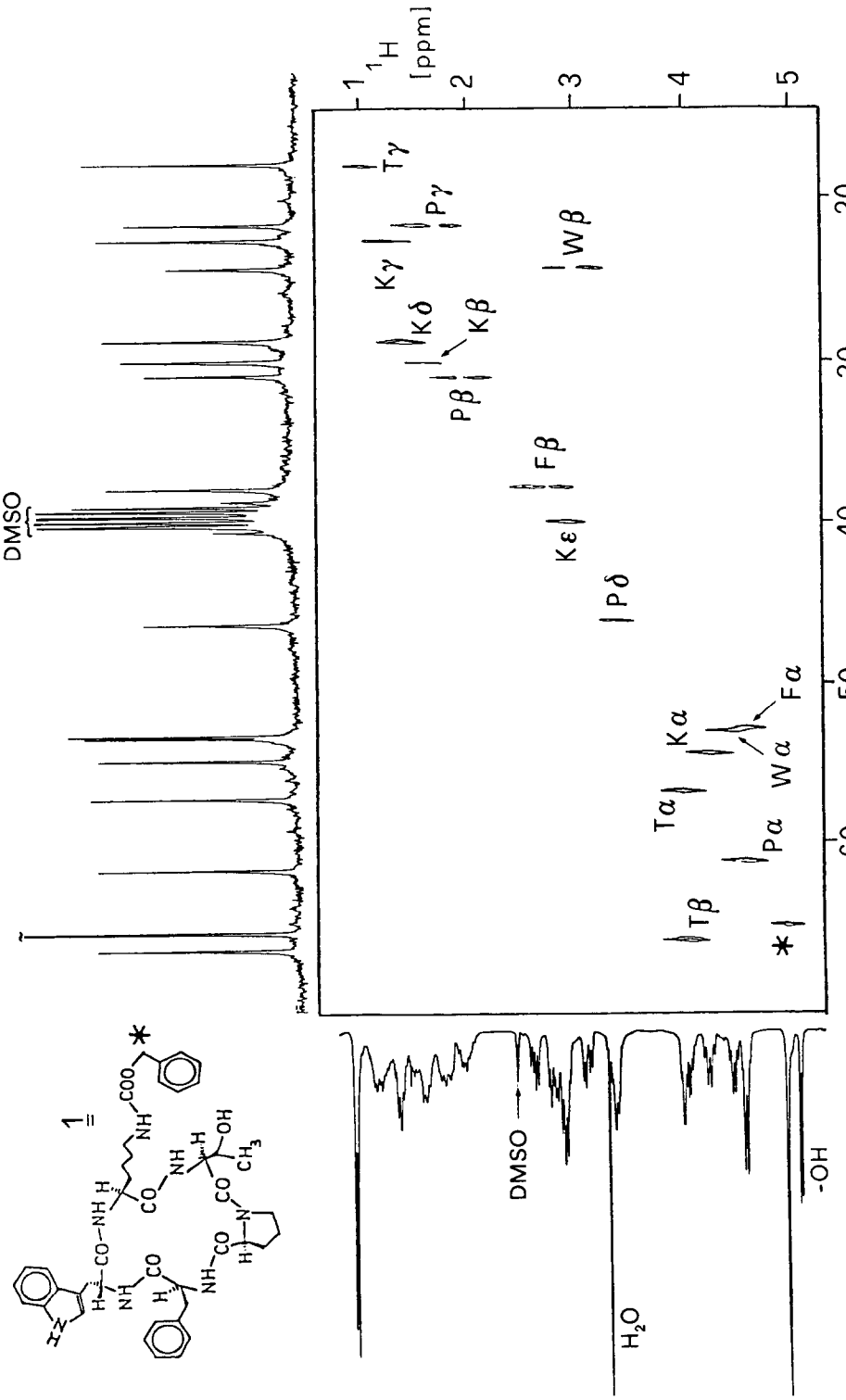

Figure 1. 2D-^{1}H-^{13}C-shift correlation of 60 mg 1 in 0.7 ml DMSO (270 MHz), total acquisition time: 15h

Fig. 1. The chemical shifts of the β- and γ-carbon atoms of Pro indicate a cis peptide bond in 1 but a trans peptide bond in 2 and 3. This is shown in Fig. 2.

The backbone conformation of 1-3 is derived mainly from ^{1}H NMR data of the amide-protons (Table 1). The temperature coefficients as well as the NH-C_{α}H-coupling constants of 3 are consistent with a β,γ-conformation (βI: D-Thr-Pro-Phe-D-Trp, γ: D-Trp-Lys-D-Thr) or a γ,γ-conformation (γ instead of βI) with internally oriented NH-groups of the two D-amino acid residues. This proposal fits well the general conformational rules derived from many cyclic pentapeptides[2].

The interpretation for 2 is similar (D-Thr-NH forms a γ turn, L-Trp-NH probably is involved in a βI turn) but the presence of an L-residue (L-Trp) in position 3 of a cyclic pentapeptide as defined in Fig. 17 in ref. 2 likely destabilizes the structure. This is represented by the relatively small values of $\Delta\delta/\Delta T$ of $2.7 \cdot 10^{-3}$ ppm of the amide-protons of Lys and Phe, and a deviation from linearity for D-Thr and Trp in the δ-T-plot above 50° C.

The relative order of temperature gradients and coupling constants of amide-protons in 1 allows a βVI turn[6] of the amino acids Lys-Thr-Pro-Phe with a cis peptide bond Thr-Pro and a γ turn of the part Phe-D-Trp-Lys

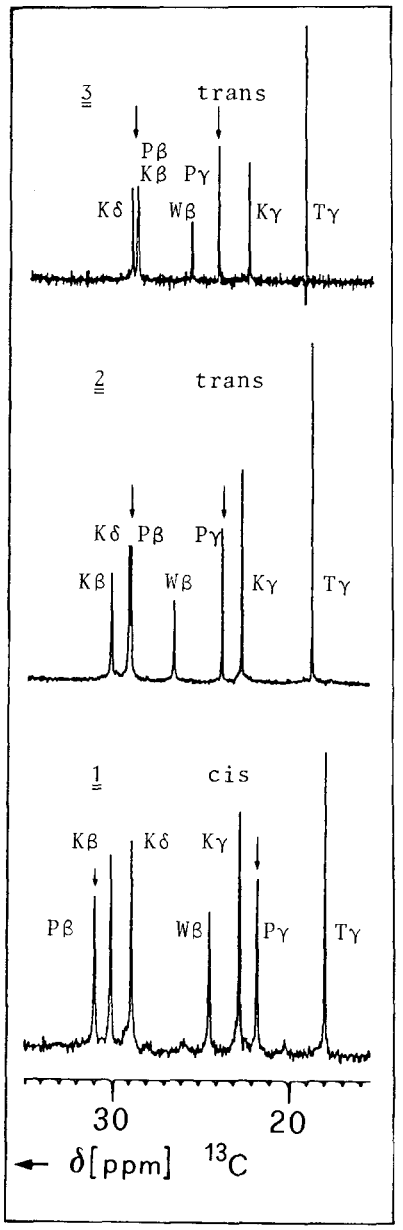

Figure 2. 67.89 MHz-^{13}C {^{1}H} NMR spectra of 1, 2 and 3 between 16 and 35 ppm[7].

Table 1. 270 MHz ^{1}H NMR data of the amide-protons of 1, 2 and 3 at 298 K in DMSO-d_6. 32 K data points

	Phe[7]	Trp[8]	Lys[9]	Thr[10]	
δ [ppm]	7.20	8.90	7.52	7.47	1
	7.28	8.17	8.07	7.95	2
	7.70	7.68	8.13	7.97	3
$\Delta\delta/\Delta T$ [$\cdot 10^{-3}$ ppm K^{-1}]	2.7	4.1	2.2	4.6	1
	2.7	1.5	2.7	0.7	2
	4.5	-1.3	4.6	1.0	3
$^3J_{HNC_\alpha H}$ [Hz]	8.4[a)]	8.5	9.8[a)]	4.0	1
	9.3[a)]	7.3	8.0	6.1	2
	8.1	6.8	8.4	7.0	3

a) NH signals covered by aromatic resonances were determined by difference spectroscopy

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7. The amino acids are symbolized by the IUPAC nomenclature: Phe = F, Lys = K, Pro = P, Thr = T, Trp = W.

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