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Conformational Behaviour of *Z*-Pro-(thr)Ox-O*Me* in Solution*

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The dipeptide *Z*-Pro-(thr)Ox-O*Me* was studied with respect to its conformation in solution using NMR spectroscopic methods. It is shown that two conformers exist at room temperature which are related to each other by a *cis-trans*—isomerization of the *Z*-Pro—peptide bond. The free energy of activation of the process is estimated.

Konformationsverhalten von Z-Pro-(thr)Ox-OMe in Lösung

Das Dipeptid *Z*-Pro-(thr)Ox-O*Me* wurde mittels Kernresonanzspektroskopie im Hinblick auf seine Konformation in Lösung untersucht. Die erhaltenen Daten weisen auf das Vorliegen von zwei Konformeren bei Raumtemperatur hin, die durch eine *cis-trans*-Isomerierung der *Z*-Pro-Peptidbindung ineinander übergehen. Die freie Aktivierungsenergie der Konformationsumwandlung konnte abgeschätzt werden.

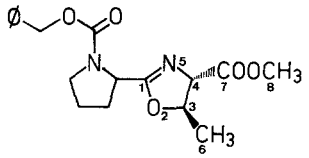
(Keywords: Peptide; Conformation; ^{13}C and 2D NMR; cis-trans-Isomerization)

Introduction

The conformational behaviour of peptides in solution has been the subject of many NMR studies in recent years. Especially the application of various 2D NMR techniques on small cyclic peptides has revealed interesting insight into structural details concerning backbone geometry, intramolecular hydrogen bonding, and the arrangement of side chains [1–12].

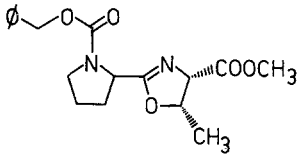
* Dedicated to Prof. Dr. *A. Neckel* on the occasion of his 60th birthday.

Peptides containing heterocyclic amino acids isolated from marine invertebrates represent a special group of substances and exhibit remarkable anticancer activities [13–16]. These compounds are of particular interest because little is known about the influence of heterocyclic structural elements on the spatial arrangement of the peptide chain [12].



Z-Pro-(thr)Ox-OMe

1



Z-Pro-allo-(thr)Ox-OMe

2

In the present paper we discuss the conformation of the dipeptide *Z*-Pro-(thr)Ox-O*Me* (**1**) in *DMSO-d_6*. It is shown that conformational rigidity is not restricted to small cyclic peptides, but can also be observed in this case.

Results and Discussion

The ^{1}H-NMR spectrum of **1** in $CDCl_3$ as well as in *DMSO-d_6* at room temperature clearly indicates the existence of two different species in solution. Because of its high boiling point *DMSO-d_6* was chosen as a solvent for the present investigation. The influence of elevated temperature on the ^{1}H NMR spectrum is shown in Fig. 1. Coalescence of corresponding signals of the two conformers can be observed at about 375 K. This fact as well as a 2 D exchange spectrum provides evidence for an interconversion of the two isomers which is relatively slow with respect to the NMR time scale. At temperatures well above the coalescence range signal averaging occurs and a clearly resolved ^{1}H NMR spectrum is observed which can be assigned in the usual way (Table 1).

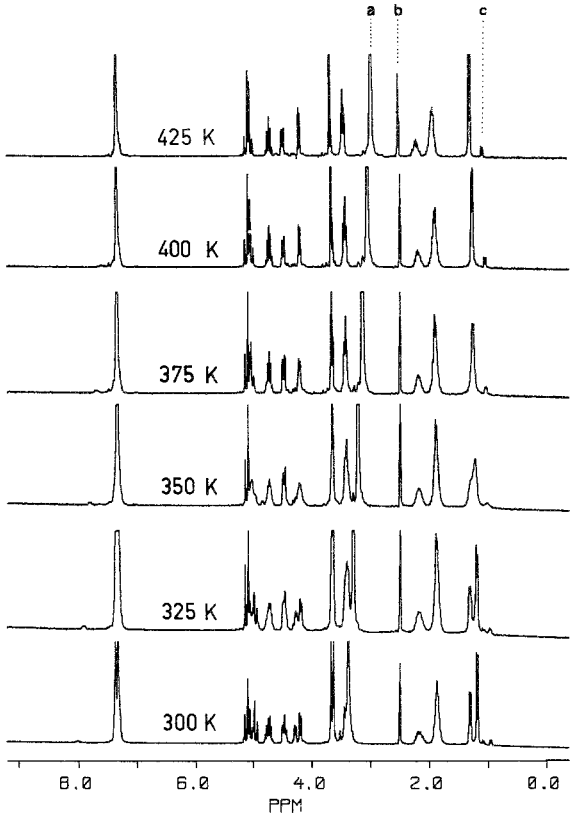


Fig. 1. Temperature dependence of the ^{1}H NMR spectrum of **1** in *DMSO-d_6*; *a* H_2O; *b* solvent; *c* impurity

Table 1. ^{1}H *chemical shifts of* **1** *in DMSO-d_6 at 425 K in ppm relative to internal TMS*

Pro-α	4.49
Pro-β	2.28/1.90
Pro-γ	1.90
Pro-δ	3.43
3	4.73
4	4.19
6	1.28
8	3.66
Ø—CH_2	5.08[a]
Ø—	7.33

[a] Center of AB-system

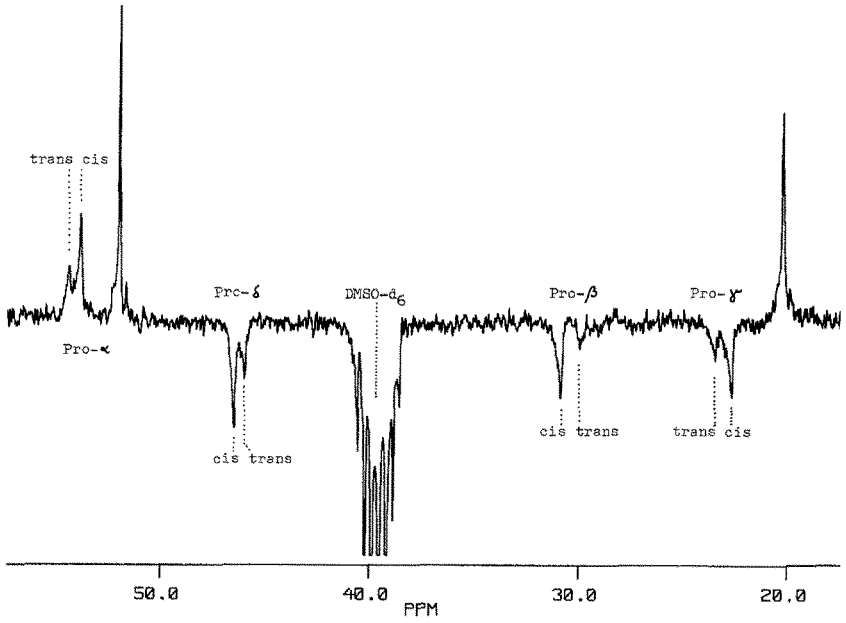


Fig. 2. Highfield region of the ^{13}C NMR spectrum of **1** in *DMSO-d_6* at 300 K with J-modulation

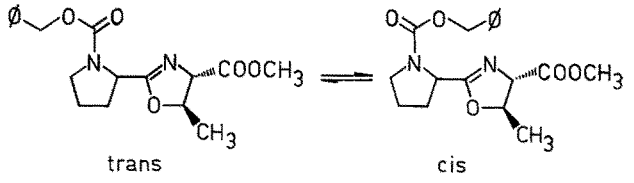


Fig. 3. *Cis-trans*-isomerization of the *Z*-Pro-peptide bond in **1**

Information on the nature of the isomerization process could be derived from the ^{13}C NMR spectrum of **1** at room temperature. The spectral region of the proline carbons (Fig. 2) exhibits a signal pattern which can be readily assigned to a mixture of *cis*- and *trans*-proline [17]. From this we conclude a conformational change involving a *cis-trans*-isomerization of the *Z*-Pro-peptide bond as shown in Fig. 3. The assignment of the remaining signals in the high field region of the ^{13}C NMR spectrum has been deduced from a 2D shift correlation experiment and is given in Table 2.

Table 2. ^{13}C *chemical shifts of* **1** *in DMSO-d_6 at 300 K in ppm relative to internal TMS*

	cis	*trans*
Pro-α	53.78	54.32
Pro-β	30.80	29.90
Pro-γ	22.61	23.40
Pro-δ	46.44	45.90
3	78.69	
4	73.53	
6	20.20	
8	51.90	
Ø—CH_2—O—	65.77	

Table 3. ^{1}H *chemical shifts of* **1** *in DMSO-d_6 at 295 K in ppm relative to internal TMS*

	cis	*trans*
Pro-α	4.47	4.43
Pro-β	2.28/1.90	
Pro-γ	1.90	
Pro-δ	3.43	
3	4.68	4.75
4	4.18	4.26
6	1.18	1.30
8	3.61[b]	3.65[b]
Ø—CH_2—O—	5.03[a, b]	5.06[a, b]
Ø—	7.30	

[a] Center of AB-system
[b] Shift values interchangeable

Using intensity information from the ^{13}C NMR spectrum, the high-field doublet of the methyl resonances of the (Thr)Ox-substructure can be assigned to the *cis*-isomer which dominates the *trans*-compound by a factor of about 1.4 at room temperature. Proceeding from this fact, a COSY experiment yields the two sets of corresponding resonances of the (thr)Ox-protons for the *cis*- and *trans*-isomer, respectively (Fig. 4, Table 3).

With the information obtained on the nature of the spatial rearrangement of **1** in *DMSO-d_6*, all phenomenological features of the ^{1}H NMR spectrum can be well understood. Molecular model considerations show

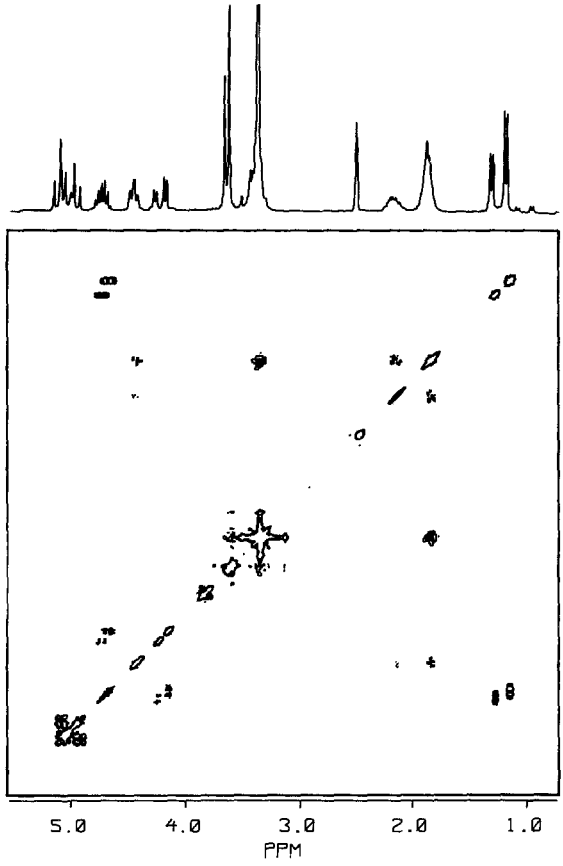


Fig. 4. 2D-COSY-NMR spectrum of **1** in *DMSO-d_6* at 295 K

an extreme exposure of the (thr)Ox-methyl group to the anisotropy cone of the aromatic ring in the *cis*-isomer. Consequently, the shift difference between the methyl doublets is large compared to those between other corresponding protons. Furthermore, the expected highfield shift of the methyl group of the *cis*-compound due to the anisotropy effect is in accordance with the fact that the *cis*-isomer is more abundant than the *trans*-isomer. Finally, we have used *Z*-Pro-allo-(thr)Ox-O*Me* (**2**) for a cross check. Compound **2** can be synthesized using *allo*-Threonine for the condensation reaction with proline and yields an oxazole ring with both methyl and ester group oriented to the "lower" side of the heterocyclus. The anisotropic shift of the methyl group should therefore be less and can indeed be found to be only 4 Hz compared to 32 Hz in compound **1** (250 MHz).

In a previous paper, we have evaluated the activation parameters of a *cis-trans*-isomerization of a proline peptide bond in a cyclic tetrapeptide [11]. As the isomerization process of compound **1** is the same except the fact that the peptide bond involved in the reaction is not part of a ring system, we attempted to gain information on ΔG^{+} using Eq. (1)* [18] with $T_c = 375\,\mathrm{K}$ and $\Delta\nu = 32\,\mathrm{Hz}$. The resulting ΔG^{+}_{375} of about $79\,\mathrm{kJ\,mol^{-1}}$ fits very well to values obtained for the cyclic compound (Fig. 5). We are well aware of the fact that the value for ΔG^{+}_{375} is only a rough

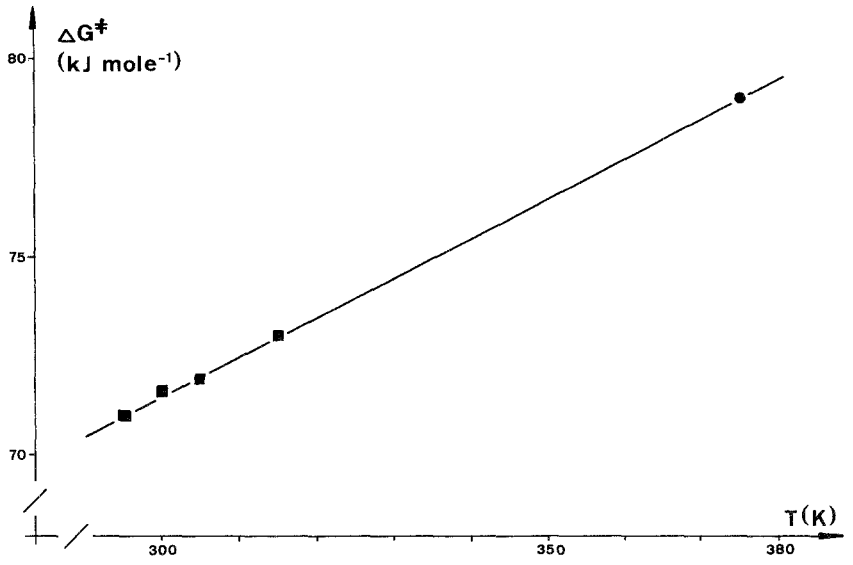


Fig. 5. Plot of ΔG^{+} vs. T. ■ values taken from Ref. [11]; ● *Z*-Pro-(thr)Ox-O*Me*

approximation, since the nonequal population of the isomers has been neglected. Nevertheless ΔS^{+} which can be derived from the slope of a ΔG^{+} vs. T plot is in good agreement with ΔS^{+} values obtained from cyclic peptides in previous investigations [19, 20]. This indicates a similar structure for the activated complex and shows that this isomerisation is predominantly determined by solvent-substrate interaction regardless if the peptide bond is part of a cyclic system or not.

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* $\Delta G^{+} = 2.3 \cdot R \cdot T_c \cdot (10.32 + \lg T_c/k_c)$; $k_c = \pi\Delta\nu/\sqrt{2}$ (1); R gas constant; T_c coalescence temperature; k_c reaction rate at T_c; $\Delta\nu$ shift difference of corresponding resonances (slow exchange limit).

Acknowledgements

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Experimental Part

All NMR spectra were recorded on a Bruker WM 250 spectrometer in connection with an aspect 2000 computer in 5 mm tubes. The temperature was adjusted using the variable temperature unit of the spectrometer. The deuterium resonance of the solvent was used for the field-frequency-stabilisation. All spectra were obtained from a 30 mg sample in 0.3 ml *DMSO-d_6*.

The 2 D experiments were performed using standard software of Bruker [21].

COSY: Pulse sequence: ($\pi/2$, ^{1}H)-(t_1)-($\pi/2$, ^{1}H)-FID; $\pi/2$-pulse: 9 μs; spectral width: 1 250 Hz in both dimensions; matrix size 128 × 512 data points; total acquisition time: 16 h. Sine-bell filtering in both dimensions and zero filling in F_1 was used before *Fourier* transformation. The spectrum was symmetrized along the diagonal.

Exchange Spectrum: Pulse sequence: ($\pi/2$, ^{1}H)-(t_1)-($\pi/2$, ^{1}H)-(τ_m)-($\pi/2$, ^{1}H)-FID with $\tau_m = 500$ ms; for all other parameters see COSY-spectrum.

^{1}H-^{13}C-Shift Correlation: Pulse sequence: ($\pi/2$, ^{1}H)-($t_1/2$)-(π, ^{13}C)-($t_1/2$)-Δ_1-($\pi/2$, ^{1}H; $\pi/2$, ^{13}C)-Δ_2-FID (BB); $\pi/2$-pulse: 17.5 μs for ^{13}C, 18 μs for ^{1}H; delays: $\Delta_1 = 7$ ms, $\Delta_2 = 3.5$ ms; spectral width: 4 100 Hz in F_2, 1 100 Hz in F_1; matrix size: 128 × 2 K data points. Exponential multiplication in F_2 and *Lorentz*-Gauss-filtering in F_1 were applied before *Fourier* transformation.

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