Journal of Peptide Science
J. Peptide Sci. **4**: 364–368 (1998)

SHORT COMMUNICATION

Trans-Cis Amide Bond Isomerization in Fulleroprolines

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Received 28 January 1998
Accepted 4 March 1998

Abstract: The ^{1}H NMR study of fulleroproline derivative Ac-Fpr-O*t*Bu and its Pro analogue Ac-L-Pro-O*t*Bu over a range of temperatures in toluene-d_8 solution has enabled the comparison of their equilibrium and activation parameters for the *trans*/*cis* interconversion around the amide partial double bond. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *trans-cis* amide equilibrium; dynamic nuclear magnetic resonance; fulleroproline; NOE differential spectroscopy; proline

We have recently reported the synthesis of fullero-3,4-proline (Fpr), a novel unnatural amino acid in which the 3,4-bond of the Pro ring is fused to a 6,6 junction of C_{60} [1]. Fpr, by far the biggest among non-protein amino acids [2], is an attractive building block for the preparation of C_{60}-based peptides and is expected to show new and interesting features. Of particular relevance are the biological properties that a number of fullerene derivatives have been shown to possess, related to the particular combination of electronic properties, hydrophobicity and bulkiness of the fullerene core [3–6]. Fpr was derivatized at the amino and carboxylic group to study its chiroptical properties [7] and incorporated in heterochiral di- and tripeptides to evaluate the propensity to induce a β-turn conformation, with relation to suitable models containing the cognated amino acid Pro [8]. It has been found that the well established tendency of Pro-containing peptides to fold in solution is matched by their Fpr counterparts. Another peculiar conformational feature of Pro is the *trans-cis* equilibrium about the -Xaa-Pro- tertiary amide bond which generates a mixture of two conformers in solution [9,10]. This rotational equilibrium is very important in the catalytic activity of the peptidyl prolyl *cis-trans* isomerase [11] and plays a central role in the rate-determining step of protein folding [10]. In this

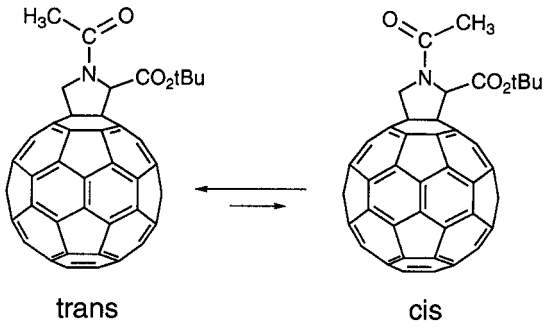

Figure 1 Rotational isomerism about the tertiary amide bond of Ac-Fpr-O*t*Bu.

Abbreviations: Fpr, fullero-3,4-proline; Ac_2O, acetic anhydride.

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CCC 1075–2617/98/050364-05$17.50

communication we calculate and discuss the thermodynamic and kinetic parameters of the *trans*-*cis* rotational isomerism of the fulleroproline derivative Ac-DL-Fpr-O*t*Bu. Enthalpy and entropy values are compared with those of the corresponding Pro-containing model compound.

Determination of the *trans*/*cis* ratio and activation parameters for the conformational equilibria of Ac-Fpr-O*t*Bu and Ac-L-Pro-O*t*Bu was performed in toluene solution by ^{1}H NMR at different temperatures.

Initially, we determined the stereochemistry of the two isomers of the Fpr derivative (Figure 1) by NOE differential spectroscopy (NOEDS) [12]. The assignment was carried out at $T = 208$ K, far below the coalescence temperature, in order to avoid saturation transfer between the corresponding signals of the conformers. The low temperature and the relevant molecular weight put the system in the negative NOE region. A relatively long saturation time (5 s) was adopted. The resulting spin diffusion was not such to alter the structural assignments. The results are reported in Figure 2. For both isomers, the δCH_2 protons resonate as doublets and the αCH proton as a singlet in the 6.7–4.5 ppm region. The acetyl methyl protons resonate at 1.96 and 1.92 ppm for the major and the minor isomer, respectively. The *t*-butyl methyl protons are undifferentiated at 1.32 ppm. Irradiation of the δCH_2 resonances of the major isomer shows cross-relaxation with the acetyl methyl protons at 1.96 ppm, while irradiation of the αCH resonance of the minor conformer displays cross-relaxation with the acetyl methyl protons at 1.92 ppm. These results were confirmed by the reverse experiments. On this basis we can unambiguously assign the structure *trans* (Figure 1) to the major isomer and the structure *cis* to the minor isomer.

During the dynamic NMR analysis we could immediately observe a different equilibrium behaviour between the Fpr derivative and the Pro model compound. The determination of the equilibrium constants was performed in the temperature ranges 298–333 K for Ac-L-Pro-O*t*Bu and 243–293 K for Ac-Fpr-O*t*Bu. The van't Hoff analysis indicates that the relative stability of the *trans* isomer is higher for the Fpr derivative than for the model, as reflected by the higher enthalpic value (Table 1). The difference between the entropic values for the two derivatives is not relevant and could be interpreted as an approximately equal solvation of the *cis* and *trans* isomers by the toluene molecules. The data relative to Ac-L-Pro-O*t*Bu are comparable with those available in literature for peptidyl-prolyl *trans*/*cis* isomerizations [13,14].

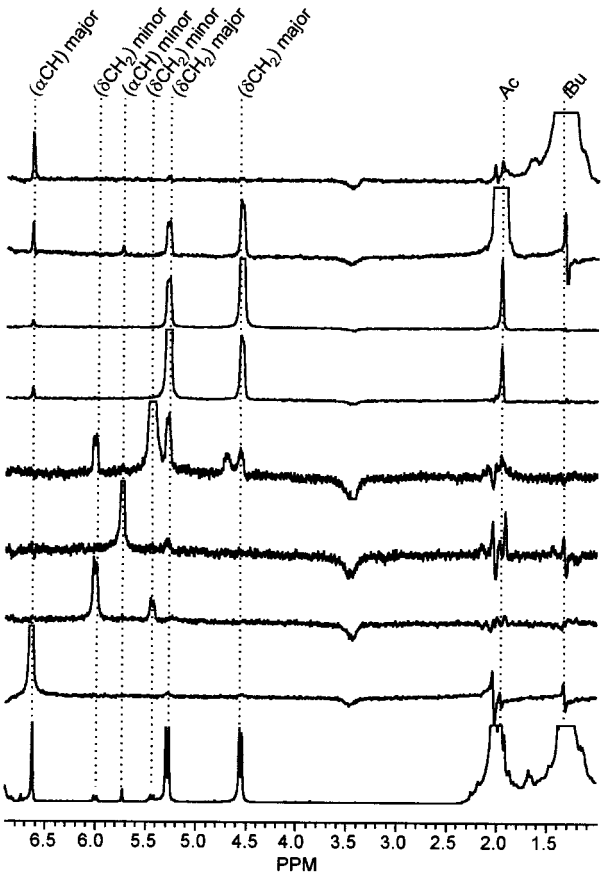

Figure 2 NOEDS analysis of the two isomers of Ac-Fpr-O*t*Bu in toluene-d_8 at 208 K. The bottom trace shows the unperturbed reference spectrum, the other traces (reported upside down) are the differentials between the perturbed spectra and the reference spectrum. The strongest differential signal is the saturated signal, also named at the top.

The activation parameters were determined in the temperature ranges 348–373 K for Ac-Pro-O*t*Bu and 283–353 K for Ac-Fpr-O*t*Bu. The rate constants at the different temperatures for the *trans*/*cis* interconversion were obtained from line-shape analysis [15] of the signals of the pyrrolidine ring protons (αCH and δCH_2) for Ac-Fpr-O*t*Bu and of those of αCH protons for Ac-L-Pro-O*t*Bu. The αCH signals of the two isomers of Ac-L-Pro-O*t*Bu were simplified to singlets by decoupling from the βCH_2 and γCH_2 signals.

The evaluation of the activation parameters was carried out with the Eyring equation [16]. The entropic values (Table 1), although oppositely signed, are relatively close to zero. Therefore, the toluene

Table 1 *cis* Isomer Content, and Thermodynamic and Kinetic Parameters for the *trans*→*cis* Process of Ac-Xaa-O*t*Bu[a]

Xaa	% *cis*[b]	$\Delta H°$ kcal mol^{-1}	$\Delta S°$ cal mol^{-1} deg^{-1}	$\Delta H^{‡}$ kcal mol^{-1}	$\Delta S^{‡}$ cal mol^{-1} deg^{-1}
L-Pro	33	0.61 ± 0.03	0.0 ± 0.1	21.2 ± 0.5	6.5 ± 0.2
Fpr	25	1.15 ± 0.06	0.9 ± 0.2	14.6 ± 0.2	-4.4 ± 0.1

[a] In toluene, concentration 50 and 15 mM for the Pro and Fpr derivatives, respectively.
[b] Measured at 298 K.

molecules do not appear to undergo any considerable rearrangement during the conformational process. Quite interesting is instead the difference of *ca* 7 kcal mol^{-1} in the activation enthalpy for the two compounds. Therefore, the rate constant of the *trans*/*cis* process at room temperature is higher for the Fpr than for the Pro derivative and the NMR 'freezing' of the conformational interconversion of the former derivative requires a temperature far below 273 K.

Figure 3 shows the resonance structures that contribute to the ground state stability of the *cis* and *trans* isomers and the transition state structure for both compounds. In the case of the Fpr derivative the lower enthalpic value indicates that the lone pair of the amide nitrogen is less available for the conjugation with the carbonyl oxygen.

This finding is in full agreement with unpublished data (A. Bagno, M. Maggini, M. Prato and G. Scorrano) which show that the basicity of fulleropyrrolidines is lower than that of the normal aliphatic amines. It may be concluded that the fullerene moiety seems to play an important role in the *trans*/*cis* isomerism by exerting a withdrawing effect on the electronic shell of the pyrrolidine nitrogen, with a consequent loss of the double-bond character of the C–N bond. Recently, it has been reported that derivatives of Pro analogues with an additional heteroatom (such as oxygen or sulphur) in the saturated heterocyclic ring also display a lowered *trans*-*cis* rotational barrier as these atoms withdraw electron density from the nitrogen [17].

EXPERIMENTAL PART

Structural and Kinetic Determinations

The analyses were performed with a Varian Unity 400 spectrometer. NOE differential spectroscopy (NOEDS) [12]: the selected multiplet was irradiated by the gated method with a 5 s cyclic perturbation of all lines with the minimal power which ensures efficient saturation. The perturbed spectra are directly subtracted at the FID level of the control spectrum (with the decoupler frequency set off-resonance).

Materials and Methods

Details regarding instrumentation used in this work for the spectroscopic characterization of the compounds have been described elsewhere [8].

Ac-L-Pro-OtBu. Ac-L-Pro-O*t*Bu [18] was synthesized from Ac_2O and H-L-Pro-O*t*Bu (the latter was prepared, in turn, by catalytic hydrogenolysis of Z-L-Pro-O*t*Bu [19]) and purified by flash chromatography on a silica gel column using $CHCl_3$ as eluant. Yield 94%. Oil. $[\alpha]_D^{20}$ $-96.3°$ (c = 0.5, MeOH). IR (film): 1737, 1652 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.41–4.34 and 4.27–4.21 (2m, 1H, Pro αCH *trans* and *cis*); 3.68–3.43 (m, 2H, Pro δCH_2 *trans*/*cis*); 2.34–1.87 (m, 4H, Pro β, γCH_2 *trans*/*cis*); 2.09 and 1.99 (2s, 3H, Ac CH_3 *trans* and *cis*); 1.47 and 1.46 (2s, 9H, O*t*Bu CH_3 *cis* and *trans*). ^{13}C NMR ($CDCl_3$): δ 171.42 (*trans*), 171.18 (*cis*), 169.45 (*cis*), 169.13 (*trans*), 81.92 (*cis*), 80.97 (*trans*), 60.75 (*cis*), 59.13 (*trans*), 47.58 (*trans*), 46.10 (*cis*), 31.23 (*cis*), 29.22 (*trans*), 27.73 (*trans*), 27.71 (*cis*), 24.43 (*trans*/*cis*), 22.56 (*cis*), 22.08 (*trans*).

Ac-DL-Fpr-OtBu. Ac-DL-Fpr-O*t*Bu was synthesized from C_{60}, para-formaldehyde, H-Gly-O*t*Bu (prepared, in turn, by catalytic hydrogenolysis of Z-Gly-O*t*Bu [19]) followed by Ac_2O in toluene [20] and purified by flash chromatography on a silica gel column using a 9:1 toluene/AcOEt mixture as eluant. Yield 11% (95% based on recovered C_{60}). IR (KBr) 1734, 1672, 1393, 1224, 1153, 575, 527 cm^{-1}. ^{1}H NMR (CS_2/$CDCl_3$ 1:1): δ 6.45 and 6.07 (2s, 1H, αCH *trans* and *cis*); 5.97 and 5.76 (2d, J = 10.7 Hz, J = 10.9 Hz, 1H, δCH_2 *cis* and *trans*); 5.49 and 5.42 (2d, J = 10.9 Hz, J = 10.7 Hz, 1H, δCH_2 *trans* and *cis*); 2.56 and 2.48 (2s, 3H, Ac CH_3 *trans* and *cis*); 1.56 and 1.50 (2s, 9H, O*t*Bu CH_3 *cis*

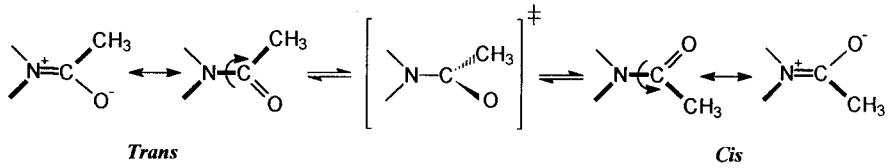

Figure 3 Resonance structures of the ground and transition states for Ac-Fpr-O*t*Bu and Ac-L-Pro-O*t*Bu.

and *trans*). ^{13}C NMR ($CS_2/CDCl_3$ 1:1): δ 169.23, 168.48, 154.58, 153.98, 152.40, 147.45, 147.34, 146.41, 146.34, 146.29, 146.17, 146.13, 146.08, 146.05, 145.71, 145.69, 145.57, 145.41, 145.37, 145.30, 145.26, 144.79, 144.48, 144.32, 143.15, 143.08, 142.74, 142.70, 142.67, 142.24, 142,10, 142.05, 141.91, 141.77, 141.61, 140.31, 139.57, 128.98, 128.23, 125.33, 83.25, 70.91, 69.81, 68.91, 59.86, 28.08, 22.39. UV-Vis (cyclohexane) λ_{max} (nm) (ε): 696 (312), 429 (2640), 311 (25200), 255 (79400), 215 (83500). FAB-MS $C_{69}H_{15}NO_3$ (MW = 905): *m*/*z* (%) 906 ($MH^{+\cdot}$, 9), 905 ($M^{+\cdot}$, 5), 720 ($C_{60}^{+\cdot}$, 100).

Acknowledgements

Part of this work was supported by CNR (Progetto Strategico Materiali Innovativi) and by MURST. The contribution of the Regione Veneto, Department for Energy and Industry, for the purchase of the NMR spectrometer is also acknowledged. The authors wish to thank Dr S. Improta and Dr A. Pastore (EMBL, Heidelberg, Germany) for preliminary 2D NMR measurements, and Dr R. Seraglia (CNR, Padova, Italy) for the FAB-MS data.

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