Pergamon

Tetrahedron Letters 40 (1999) 7987–7991

TETRAHEDRON LETTERS

Influence of *cis–trans* isomerisation on pentapeptide cyclisation

Cédric Sager, Manfred Mutter and Pascal Dumy *,†

Institute of Organic Chemistry, University of Lausanne, Lausanne, Switzerland

Received 20 July 1999; accepted 31 August 1999

Abstract

Cyclisation reactions on pentapeptides containing the turn promoting residues Gly and Pro are investigated. Although the cyclisation reaction is fast, solvent-dependent mixtures of cyclic penta- and decapeptides are observed. NMR studies suggest a strong dependence of the monomer/dimer ratio on the *cis–trans* isomerisation found in linear and cyclic peptides. The critical influence on ring closure of a *cis* Xaa–Pro peptide bond conformation is demonstrated by using the *cis* peptide bond-inducing residue 2,2-dimethyl-1,3-thiazolidine-4 carboxylic acid (Dmt, pseudo-proline) as proline substitute. The results shed new light on the role of *cis–trans* isomerisation in pentapeptide cyclisation.

Keywords: peptide cyclisation; *cis–trans* isomerisation; conformation.

Small cyclic peptides are of great interest for the elucidation of bioactive conformations due to their restricted conformational flexibility. The readiness of a linear precursor to cyclise depends on the size of the ring to be closed, and generally no difficulty arises for the cyclisation of peptides containing more than six amino acids. For penta- and hexapeptides, it is now well known that the presence of turn-inducing amino acids such as proline or glycine as well as D-configurated or *N*-alkylated residues[1] within the linear peptide sequence enhance the tendency for cyclisation. Obviously, cyclisation depends on the propensity of the linear precursor to adopt a conformation similar to the transition state required for cyclisation,[2] resulting in a conformationally controlled reaction. In view of these general findings, the role of *cis–trans* isomerisation should have a pronounced impact on cyclisation reactions of short peptides. In order to elucidate these effects, we investigate here the conformational preferences of linear and cyclic peptides by NMR spectroscopy. The influence of *cis*-amide bond conformation on peptide cyclisation was studied by incorporating the *cis*-inducing proline surrogate 2,2-dimethyl-1,3-thiazolidine-4 carboxylic acid (Dmt, pseudo-proline).

The cyclopentapeptides were designed for their use as topological templates in protein de novo design.[3] The linear peptides **1–3** (Table 1) were assembled using standard Fmoc solid-phase chemistry[4] with the highly acid-labile linker unit, the Sasrin handle.[5] For peptide **3**, the pseudo-proline unit Dmt was

* Corresponding author. Tel: +33 76 63 55 45; fax: +33 76 51 43 82; e-mail: pascal.dumy@ujf-grenoble.fr

† Present address: LEDSS-5, Université Joseph fourier et CNRS, Grenoble, France.

0040-4039/99/$ - see front matter
PII: S0040-4039(99)01671-8

Table 1

	Peptides	mM[1]	a/b[2]	Cis (%)[3]		
1	K(Dde)-P-G-K(Dde)-G	1	49/51	**1** (17)	**1a** (80)	**1b** (<1)
		0.1	87/13			
		1[4]	0/100			
2	A-P-G-K(Dde)-G	1	26/74	**2** (21)	**2a** (83)	**2b** (<1)
		0.1	67/33			
3	K(Dde)-Dmt-G-K(Dde)-G	1	100/0	**3** (> 99)	**3a** (> 99)	---------

[1]Peptide concentration used for cyclisation. [2]Monomer/dimer ratio observed during the cyclisation of linear peptides **1–3** in DMF. [3]*cis*-Amide bond content as determined by NMR in DMSO-d_6. [4]Cyclisation of **1** carried out in CH_2Cl_2.

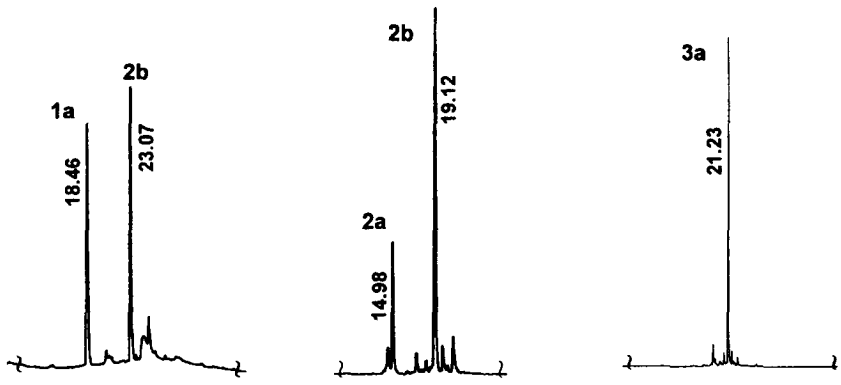


Figure 1. HPLC profiles of the cyclisation reactions observed in DMF (1 mM) for peptides **1**, **2** and **3**; **a**=monomer, **b**=dimer

incorporated as Fmoc-K(Dde)-Dmt-OH as reported.[6] All peptides were obtained in excellent yields and high purity and were characterised by ESI-MS and NMR. Their cyclisations were effected with PyBOP[7] in DMF at high dilution.[8] Gly was chosen as the C-terminal residue to exclude any epimerisation problem as well as to minimise cyclodimerisation.[9]

In agreement with the presence of the turn promoting residues Pro and Gly, the cyclisation of peptide **1** and **2** were fast (within a few minutes) as monitored by the disappearance of the HPLC-peaks of the linear peptides. Surprisingly, a mixture of two cyclic products **1a** and **1b** or **2a** and **2b** (Table 1, Fig. 1) was isolated from the cyclisation reaction.

Careful analysis by electrospray mass spectrometry revealed that **1a** and **2a** correspond to the expected cyclopentapeptides, whereas **1b** and **2b** correspond to the cyclodimers.[10] Neither lowering the peptide concentration (Table 1) nor the use of PyAOP as coupling reagent prevented the cyclodimer formation. Remarkably, when the cyclisation of **1** was carried out in CH_2Cl_2 the cyclodimer **1b** was obtained exclusively. In addition, the presence of the linear dimer intermediate could not be observed during any of the reactions studied here indicating a fast cyclodimerisation step.

The incorporation of glycine or proline as turn-inducing elements is generally considered as a prerequisite for ring closure of short peptides,[11] proline having the highest positional preference in reverse turns,[12] and glycine playing the role of a L- or D-amino-acid, thus accommodating the *i*+1 or the *i*+2 position of a turn, respectively.[13] Both residues presumably increase the propensity of the linear precursor to adopt a conformation similar to the transition state required for cyclisation. Based on these considerations, the cyclodimer ratios found for **1** and **2** were unexpected. Since cyclodimerisation is observed only for peptides containing proline which readily exhibits *cis–trans* isomerisation around the Xaa–Pro peptide bond, the cyclisation reaction should strongly depend on the *cis–trans* ratio. To probe

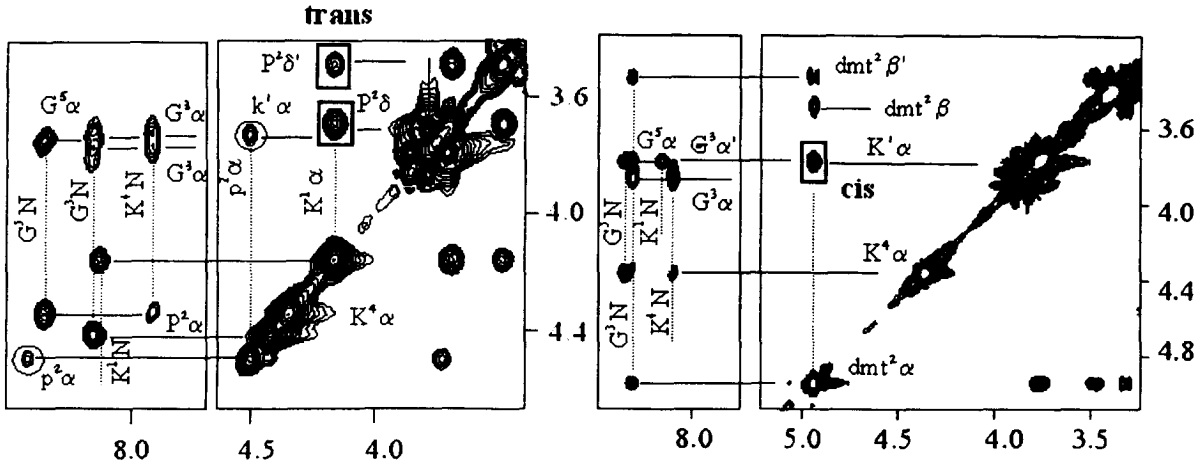


Figure 2. 2D-ROESY spectra (DMSO-d_6, 400 MHz, τ_m=200 ms) of linear **1** (left, lower cases indicate the minor conformation) and **3** (right)

this hypothesis, a 2,2-dimethyl-1,3-thiazolidine-4 carboxylic acid residue (Dmt) was incorporated in place of proline for the induction of a *cis* peptide bond.[14] Moreover, the structural similarity of Dmt with Pro allows modelling of the *cis* form about the Lys–Pro bond of the linear peptide **1** without additional steric hindrance. As shown in Fig. 1, the cyclisation of peptide **3** containing Dmt resulted exclusively in the desired cyclopentapeptide **3a**.

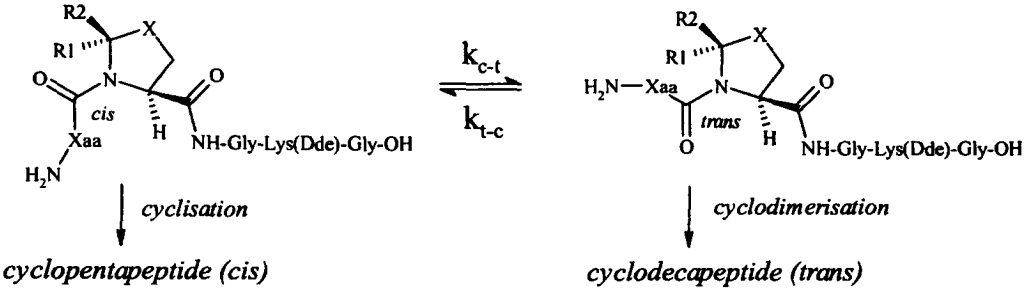
The proline-containing peptides were found by NMR to adopt at least two distinct conformations due to the *cis–trans* isomerism about the Lys–Pro (**1**, **1a**, **1b**) or Ala–Pro (**2**, **2a**, **2b**) peptide bond.[15] These species were characterised by the presence of two different sets of signals in the ^{1}H NMR spectrum and exchanged cross peaks between their proton resonances in the 2D ROESY spectra. The results obtained for the different peptides are depicted in Table 1. A major all-*trans* conformation was observed for the linear peptides **1** and **2**. Analysis of ROE cross peaks, *J* coupling constants as well as NH amide temperature coefficients did not reveal a particular set of conformation. Similarly, the cyclodimer **1b** and **2b** also adopted an all-*trans* conformation close to those found for similar cyclodecapeptides.[16] Conversely, for the cyclopentapeptides **1a** and **2a**, Lys–Pro or Ala–Pro amide bonds were predominantly *cis* (80–83%) as inferred by strong ROE between the corresponding α protons. As expected, linear **3** and cyclic **3a** peptides showed a very high *cis* Lys–Dmt amide bond content (>99%). A comparison of the 2D-ROESY spectra obtained for **1** and **3** is shown (Fig. 2).

Moreover, a comparison of the NMR data obtained for the major *cis* conformation of **1a** and **3a** revealed similar structural properties indicating that the Dmt residue efficiently reproduces the major peptide conformation of **1a**.

Cyclic pentapeptides are considered as the smallest ring size allowing the formation of an all-*trans* amide conformation without large energy penalties[17] as verified for the cyclisation of an analogue of **1** containing Ala in place of Pro (not shown). However, the presence of a *cis* Xaa–Pro conformation in **1a** and **2a** can be rationalised in view of the higher *cis–trans* isomerisation rate in Pro containing peptide. High *cis* contents were also reported for cyclopentapeptide related to elastin[18] or integrin ligands.[19]

In our case, the conformation of the transition state of the ring closure reaction corresponded closely to that of the cyclic product. For the peptide containing Dmt, a quasi-cyclic transition state containing a *cis* amide bond in the linear peptide **3** was attained without energy expense due to the presence of the Dmt residue, resulting in a complete shift of the equilibrium to the left in Scheme 1 ($k_{t-c} \gg k_{c-t}$). The situation is more complex for peptides containing Pro because the cyclisation of the *cis* and *trans* conformation of

the linear peptides may lead to different products. Ring closure of the *cis* conformation led to the desired cyclopentapeptide while the *trans* form was more prone to cyclodimerisation (Scheme 1). Apparently, the predominance of the *trans* form in the linear peptides ($\Delta G°_{c-t} \approx -1$ kcal/mol) is sufficient to compensate the bimolecular dependence of the dimerisation process. Moreover, if the cyclisation of the dimer is the fastest step, the *cis–trans* equilibrium is perturbed in turn to afford only the cyclodimer. This might be the case for the cyclisation of **1** performed in CH_2Cl_2. Unfortunately, the very broad signals observed by NMR for **1** and **1a** in CD_2Cl_2 precluded any consistent measurement of the *cis–trans* ratio. However, Urry et al.[18] observed for the closely related elastin analogue cyclo(Val-Pro-Gly-Val-Gly) an increase of the *trans* form in going from water to DMSO. Additionally, it is also well known that the *cis–trans* isomerisation barriers are lowered with decreasing solvent polarity.[20]



Scheme 1. Influence of *cis–trans* isomerisation of the Xaa–Pro amide bond on the cyclisation reactions of peptides **1–3**

In conclusion, the cyclisation of pentapeptides containing Gly and Pro proceeds readily but cyclodimerisation reactions may occur competitively. Our data indicate that this side reaction depends on the *cis–trans* equilibrium of the Xaa–Pro bond. *Cis*-amide bond-inducing residue (Dmt) proves to be a versatile tool to enforce and direct cyclisation reactions in short peptides.

Acknowledgements

This work was supported by the Swiss National Science Foundation.

References

1. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831–8838.
2. Schmidt, U.; Langner, J. *J. Peptide Res.* **1997**, *49*, 67–73.
3. (a) Dumy, P.; Eggleston, I.; Cervigny, S.; Sila, U.; Sun, X.; Mutter, M. *Tetrahedron Lett.* **1995**, *36*, 1255–1258. (b) Tuchscherer, G.; Scheibler, L.; Dumy, P.; Mutter, M. *Biopolymers (Peptide Science)* **1998**, *47*, 63–73.
4. Stewart, J.; Young, J. In *Solid Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, 1989.
5. Mergler, M.; Tanner, R.; Gosteli, J.; Gregg, P. *Tetrahedron Lett.* **1988**, *29*, 4005–4008.
6. Wöhr, T.; Wahl, F.; Nefzi, A.; Rohwedder, B.; Sato, T.; Sun, X.; Mutter, M. *J. Am. Chem. Soc.* **1996**, *118*, 9218–9227.
7. Coste, J.; Le Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205–208.
8. A typical procedure was as follows: To a solution of **1** (81 mg, 0.10 mmol) and PyBOP (52 mg, 0.10 mmol) in DMF (100 ml) a solution of DIPEA (1 M) in DMF (≈0.3 ml) was added at room temperature until a pH value of about 8–9 was obtained. The cyclisation was monitored by analytical reversed phase HPLC until complete disappearance of the linear peptide **1** (within a few minutes). After removal of the solvent under reduced pressure, the crude product was precipitated by Et_2O and purified by preparative reversed phase HPLC.
9. Kopple, K. D. *J. Pharmac. Sci.* **1972**, *61*, 1345–1356.

10. Confirmation of dimer formation was proven by the presence of characteristic ESI-MS peaks, i.e. $[M+H]^+$ and $[M+3H]^{3+}$ although the major observed peak was the $[M+2H]^{2+}$.
11. Kessler, H.; Haase, B. *Int. J. Pept. Protein Res.* **1992**, *39*, 36–40.
12. Chou, P.; Fasman, G. *J. Mol. Biol.* **1977**, *115*, 135–175.
13. (a) Ramanchandra, G. N.; Chandrasekaran, S. In *Progress in Peptide Research*; Lande, S., Ed.; Gordon and Breach: New York, 1972; Vol. 2, pp. 195–215. (b) Rose, G. D.; Gierash, L. M.; Smith, J. A. *Crit. Rev. Biochem.* **1985**, *18*, 1–109.
14. Dumy, P.; Keller, M.; Ryan, D. E.; Rohwedder, B.; Wöhr, T.; Mutter, M. *J. Am. Chem. Soc.* **1997**, *119*, 918–925.
15. NMR spectra recorded in deuteriated DMF gave similar results.
16. Dumy, P.; Eggleston, I. M.; Esposito, G.; Nicula, S.; Mutter, M. *Biopolymers* **1996**, *39*, 297–308.
17. Ramakrishnan, C.; Sarathy, K. P. *Int. J. Protein Res.* **1969**, *1*, 63–71.
18. Khaled, M. A.; Venkatachalam, C. M.; Sugano, H.; Urry, D. W. *Int. J. Peptide Protein Res.* **1981**, *17*, 23–33.
19. Viles, J. H.; Mitchell, J. B. O.; Gough, S. L.; Doyle, P. M.; Harris, C. J.; Sadler, P. J.; Thornton, J. M. *Eur. J. Biochem.* **1996**, *242*, 352–362.
20. Stein, R. L. *Adv. Protein Chem.* **1993**, *44*, 1–24.