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β-Turn modulation by the incorporation of c_6Ser into Xaa-Pro dipeptide

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Abstract—The enantiomerically pure (1*R*,2*S*)- and (1*S*,2*R*)-1-amino-2-hydroxycyclohexane-1-carboxylic acids (c_6Ser) **1** and **2** were incorporated into dipeptide Xaa-Pro by a resolution method, involving the aminolysis of a racemic 2-*tert*-butyl-5(4*H*)-oxazolone intermediate with the hydrochloride derivative of L-Pro-NHMe. Once the model dipeptides Piv-(1*R*,2*S*)-c_6Ser-L-Pro-NHMe (**6**) and Piv-(1*S*,2*R*)-c_6Ser-L-Pro-NHMe (**7**) were formed and separated, their X-ray analysis allowed the characterisation of a non-folded structure for peptide **6** and an unexpected type I β-turn with a *trans*-Pro at the i+2 position for peptide **7**.

Over the last years, the synthesis of new molecules to imitate biological events is a key goal in the chemistry field. In this area, peptidomimetics[1–4]—compounds that can mimic or block the natural effect of the peptide at the receptor level—are the driving force of the work of many chemists. The extraordinary advance in the synthesis of new non-proteinogenic α,α-disubstituted amino acids[5,6] allows the development of peptidomimetics, since when these amino acids are incorporated into peptides they confer attractive features to these new biomolecules.

The introduction of bridges connecting different fragments of the molecule provides an exceptional method to incorporate some interesting properties as well as to stabilise β-turn conformations,[7–11] a secondary structure controlled by the torsional angles ϕ and ψ and a prerequisite for the formation β-hairpin structure.[12]

The sequence Xaa-Pro has been studied, mainly the *cis*/*trans* isomerisation of the tertiary amide bond, in solution state by several methods.[13,14] Nevertheless, there are not many examples of solid state analysis.[15,16]

The dipeptide Ser-Pro is a good target to introduce restricted α-amino acids due to the important role that L-serine plays in peptides[17] and to the biological activity shown by some of its derivatives.[18]

In this context, c_6Ser has received extensive attention due to the high rigidity achieved in this molecule and several approaches have obtained the serine analogues *cis* and *trans*-c_6Ser, both as racemic and as chiral forms.[19–25] In this paper, we introduce the *cis*-c_6Ser **1** and **2** (Fig. 1) into dipeptide Xaa-Pro to obtain both isomers of Piv-*cis*-c_6Ser-L-Pro-NHMe.

The use of coupling reagents is the most popular technique for the peptide synthesis with bulky α,α-disubstituted amino acids, due to the problems arising from the space congestion that inhibits an efficient peptide bond formation.[26] However, oxazolones are themselves acylating agents and can undergo aminolysis and provide an excellent alternative for amide bond creation.[27–29] Therefore, 2-alkyl-5(4*H*)-oxazolone has been

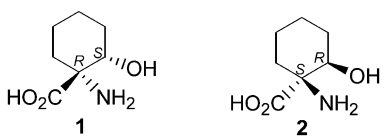

Figure 1. Structures of (1*R*,2*S*)-c_6Ser and (1*S*,2*R*)-c_6Ser.

Keywords: amino acids and derivatives; cyclohexanes; oxazolones; resolution; peptide analogues/mimetics; X-ray crystal structures.

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used as reagent for activating and coupling amino acids.[30]

With this aim, we synthesised 2-*tert*-butyl-5(4*H*)-oxazolone *rac*-**5** from trifluoroacetic salt *rac*-**3**, which was obtained from methyl 2-benzamidoacrylate following our synthetic route.[25] Thus, *rac*-**3** was acylated with pivaloyl chloride (PivCl) in the presence of diisopropylethylamine (DIEA) and the further hydrogenation of the double carbon–carbon bond allowed to obtain the compound *rac*-**4**. The methyl ester group of *rac*-**4** was hydrolysed with LiOH to the corresponding carboxylic acid and the oxazolone formation was carried out using *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoroborate (TBTU) in acetonitrile (Scheme 1).

The coupling reaction between the 5(4*H*)-oxazolone *rac*-**5** and the commercially available hydrochloride derivative of L-Pro-NHMe was carried out in the presence of DIEA, at room temperature, to yield the mixture of diastereoisomers Piv-(1*R*,2*S*)-c_6Ser-L-Pro-NHMe (**6**) and Piv-(1*S*,2*R*)-c_6Ser-L-Pro-NHMe (**7**), which were separated by a silica gel column chromatography, eluting with MeOH–EtOAc (1:9) (Scheme 1).

We obtained single crystals for compounds **6** and **7** by slow evaporation of a chloroform/ether mixture, which were subject to X-ray diffraction analysis. The absolute configuration of the c_6Ser residue was recognised as (1*R*,2*S*) in **6** and (1*S*,2*R*) in **7**, taking L-proline as a reference.

The diagram of **6**† (Fig. 2) shows a non-folded structure with an intramolecular hydrogen bond between the OH of c_6Ser and CONHMe (C=O···H-O distance = 1.91 Å).

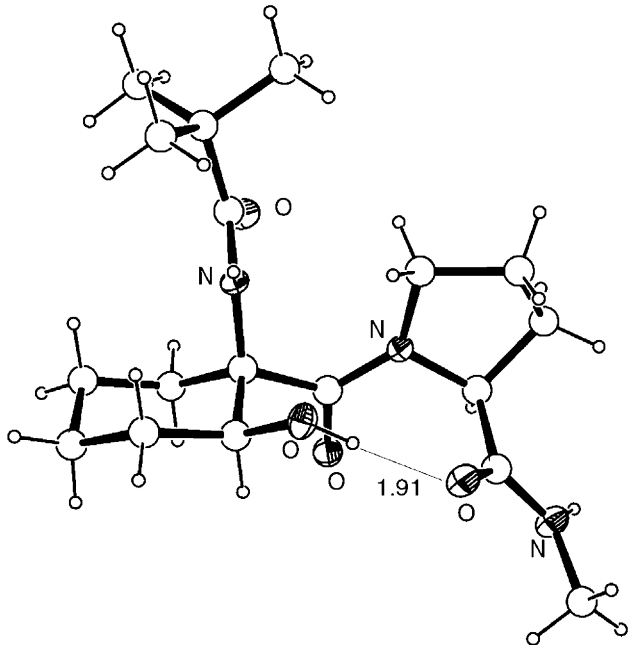
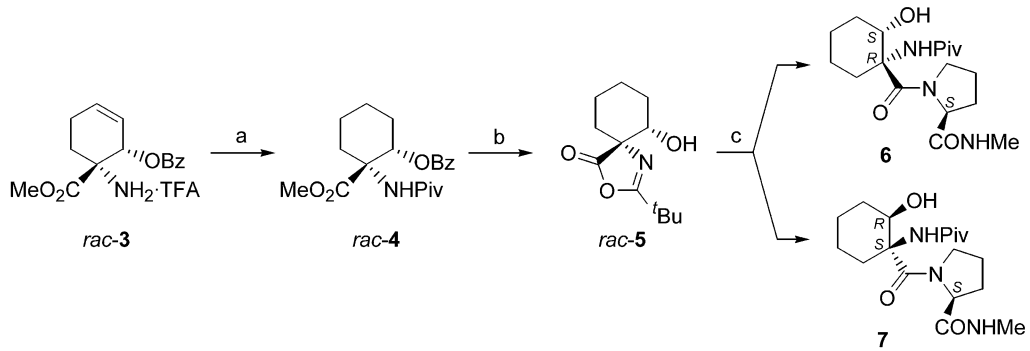


Figure 2. Crystal molecular structure of **6**.

However, for the dipeptide **7**,‡ the ORTEP diagram shows a β-turn conformation stabilised by a NHMe to CO′Bu intramolecular hydrogen bond (C=O···H–N distance = 1.99 Å) and with an $\alpha C_i \rightarrow \alpha C_{i+3}$ distance of 5.72 Å (this distance should be <7 Å for a typical β-turn). The torsion angles are summarised in Table 1. These data correspond to the ideal type I β-turn. In both cases, dipeptides **6** and **7**, the proline residue adopts a *trans* disposition (Fig. 3).



Scheme 1. (a) (i) PivCl, DIEA, CH_2Cl_2, rt, 81%, (ii) H_2/Pt–C, EtOAc, rt, 100%; (b) (i) LiOH·H_2O, MeOH, rt, 88%, (ii) TBTU, DIEA, CH_3CN, rt, 81%; (c) (i) L-Pro-NHMe·HCl, DIEA, CH_3CN, rt, 82%, (ii) separation by column chromatography.

† Crystal data for **6**: (a) $C_{18}H_{31}N_3O_4$, $M_w = 353.46$, colourless prism of 0.6×0.35×0.2 mm, $T = 123$ K, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 9.2889(2)$, $b = 9.4421(2)$, $c = 21.2078(6)$ Å, $V = 1860.08(8)$ Å^3, $D_{calcd} = 1.262$ g cm^{-3}, $F(000) = 768$, $\lambda = 0.71073$ Å (Mo Kα), $\mu = 0.089$ mm^{-1}, Nonius kappa CCD diffractometer, θ range 2.36–27.88°, 10007 collected reflections, 4388 unique, full-matrix least-squares (SHELXL-97[a]), $R_1 = 0.0443$, $wR_2 = 0.1043$ ($R_1 = 0.0538$, $wR_2 = 0.1096$ all data), goodness-of-fit = 1.034, residual electron density between 0.278 and –0.324 e Å^{-3}. Hydrogen atoms were located from mixed methods (electron-density maps and theoretical positions). Further details on the crystal structure are available on request from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, UK on quoting the depository number 174552.
[a] Sheldrick, G. M. SHELXL-97. Program for the refinement of crystal structures. University of Göttingen, Germany, 1997.

‡ Crystal data for **7**: $C_{18}H_{31}N_3O_4$, $M_w = 353.46$, colourless prism of 0.32×0.25×0.18 mm, $T = 126$ K, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 11.3725(4)$, $b = 11.9710(4)$, $c = 13.9398(6)$ Å, $V = 1897.88(12)$ Å^3, $D_{calcd} = 1.237$ g cm^{-3}, $F(000) = 768$, $\lambda = 0.71073$ Å (Mo Kα), $\mu = 0.087$ mm^{-1}, Nonius kappa CCD diffractometer, θ range 2.24–27.88°, 11655 collected reflections, 4502 unique, full-matrix least-squares (SHELXL-97[a]), $R_1 = 0.0466$, $wR_2 = 0.1024$ ($R_1 = 0.0671$, $wR_2 = 0.1114$ all data), goodness-of-fit = 1.023, residual electron density between 0.221 and –0.193 e Å^{-3}. Hydrogen atoms were located from mixed methods (electron-density maps and theoretical positions). Further details on the crystal structure are available on request from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, UK on quoting the depository number 174553.
[a] Sheldrick, G. M. SHELXL-97. Program for the refinement of crystal structures. University of Göttingen, Germany, 1997.

Table 1. Main torsion angles for the Ser-Pro derivatives by X-ray diffraction

	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}	ω_1	ω_2	ω_3
Piv-(1*R*,2*S*)-c_6Ser-L-Pro-NHMe	71.1	22.2	−70.6	165.3	168.5	162.7	178.3
Piv-(1*S*,2*R*)-c_6Ser-L-Pro-NHMe	−59.8	−21.5	−73.1	−14.5	−172.4	175.3	−177.3
L-Ser-L-Pro[a]	−81	152	−82	173	−177	163	−170
L-Ser-L-Pro[b]	−48.5	133.6	−88.1	8.2			
Ideal type I β-turn[c]	−60	−30	−90	0			

[a] Dipeptide L-Ser-L-Pro extracted from Cycloleonuripeptide D shows a non-folded structure, Ref. 16.
[b] Dipeptide L-Ser-L-Pro extracted from Stylopeptide 1 shows a type VIa1 β-turn (*cis* conformation of the proline residue) Ref. 15.
[c] Ref. 31.

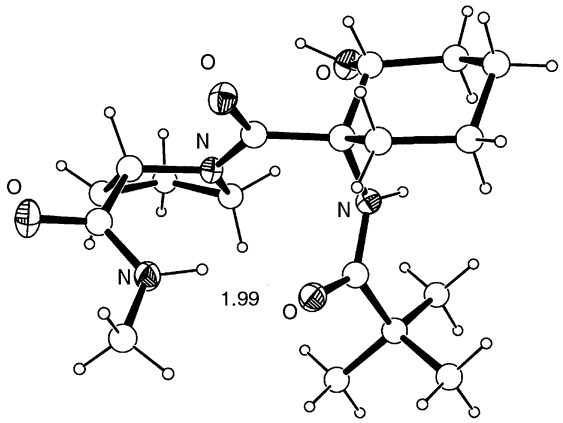


Figure 3. Crystal molecular structure of **7**.

In conclusion, we have verified in solid state that the strain serine *cis*-c_6Ser is capable to induct a type I β-turn or a non-folded structure in the dipeptide Xaa-Pro, depending on its absolute configuration, fixing the *trans* amide bond for the proline residue. Therefore, an unexpected isolated β-turn with a Pro at the i+2 position is observed. The significance of this feature is mainly due to the fact that it is well-known that different β-turns are induced by Pro at the i+1 or i+2 position, with the additional condition that this residue must be a *cis*-Pro. Nevertheless, there are few examples reported on β-turns with a *trans*-Pro at the i+2 position[31–34] (Fig. 4).

Further studies to incorporate other cyclohexane serine analogues into Xaa-Pro dipeptide as well as to evaluate the preservation of these conformational features in solution are in progress.

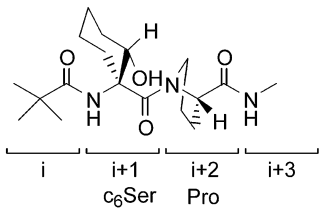


Figure 4. Identification of the residues i, i+1, i+2 and i+3 in the β-turn of peptide **7**.

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