The Synthesis and Conformational Analysis of a Pair of Diastereoisomeric Cyclic Peptides with *cis* and *trans* Amide Bonds, Respectively

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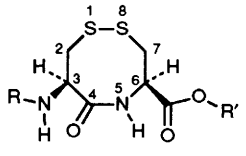
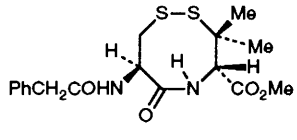
N-Protected, conformationally constrained, eight-membered ring containing cyclic dipeptides derived from methyl cyclo-[(*R*)-cysteinyl-(*R*)-penicillamine] contain a *cis* amide bond, whilst the corresponding peptides derived from cyclo-[(*R*)-cysteinyl-(*S*)-penicillamine] contain a *trans* amide bond.

One of the most common ways of preparing conformationally constrained peptides for the investigation of structure–activity relationships is the use of disulfide bonds to form a cyclic peptide derivative.[1] Despite this, no systematic investigation of conformations adopted by peptides containing disulfide rings of various sizes has been carried out. The simplest such compounds, are the cyclo-[cysteinyl-cysteine] derivatives which contain an eight-membered ring. It is known from X-ray crystallographic studies that cyclo-[(*R*)-cysteinyl-(*R*)-cysteine] **1**, and methyl *N*-Boc-cyclo-[(*R*)-cysteinyl-(*R*)-cysteine] **2** contain a *cis* amide bond within the eight-membered ring,[2] whilst methyl *N*-phenacyl-cyclo-[(*R*)-cysteinyl-(*S*)-penicillamine] **3** contains a *trans* amide bond within the eight-membered ring;[3] (Boc = *tert*-butoxycarbonyl). No explanation for this difference in amide bond geometry has been reported, nor is it known whether the solution conformations of these peptides are the same as the solid state structures. Recently, the conformation of a heptapeptide containing a cyclo-[(*R*)-cysteinyl-(*R*)-cysteine] unit has been investigated by NMR.[4] The compound was found to adopt two conformations differing in the amide bond geometry within the eight-membered ring; the major conformer having a *cis* amide bond, and the minor conformer a *trans* bond.

Hence, it appears that the preferred geometry of an amide bond within an eight-membered ring is determined by a variety of factors, including substituents and the relative configuration of the chiral centres within the ring. By studying the conformations adopted by such compounds, it should be possible to obtain a better understanding of the factors that determine peptide conformation in general. In this communication, we determine the conformation of a range of eight-membered ring containing dipeptides in chloroform solution, and investigate the factors that affect the conformation adopted by these compounds.



1; R = H, R' = H
2; R = Boc, R' = Me



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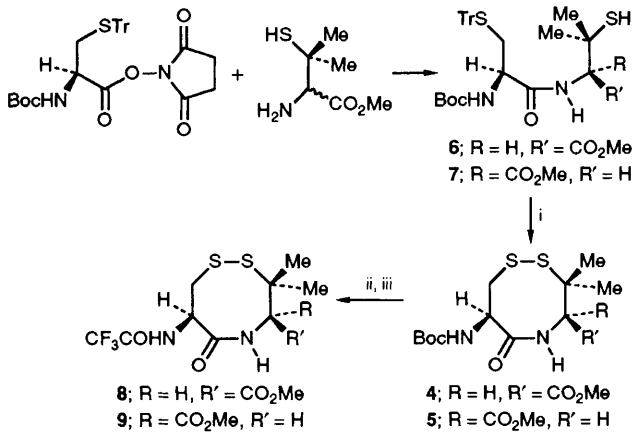
† Work carried out whilst a visiting fellow at the University of Wales, Bangor from University of Padua, Italy.



Scheme 1 *Reagents*: i, I_2–MeOH; ii, CF_3CO_2H; iii, $(CF_3CO)_2O$ (Tr = trityl)

Comparing structures **2** and **3**, it seemed that one or more of three factors could be responsible for the change in amide bond geometry; the differing amine protecting group, the presence of two methyl groups in the penicillamine residue of compound **3**, or the differing configuration at the C-terminal amino acid (C-6) of compounds **2** and **3**. In order to investigate the relative importance of these factors, a series of derivatives differing in only one of these ways have been prepared. Thus, the diastereoisomeric compounds methyl *N*-Boc-cyclo-[(*R*)-cysteinyl-(*R*)-penicillamine] **4**‡, and methyl *N*-Boc-cyclo-[(*R*)-cysteinyl-(*S*)-penicillamine] **5** were prepared as shown in Scheme 1. Coupling of *N*-Boc-*S*-trityl-(*R*)-cysteine-*N*-hydroxysuccinimide ester[5] with methyl (*R*)- or (*S*)-penicillamine[6] gave the linear dipeptides **6** and **7** which were cyclised by treatment with iodine in methanol under high dilution,[7] to give the desired cyclic peptides **4** and **5**.

The conformations of peptides **4** and **5** were investigated using NMR spectroscopy and the nuclear Overhauser effect (NOE). Compounds **4** and **5** exist in chloroform solution as a mixture of two conformations which interconvert on the NOE timescale, hence the NOE experiments were conducted at low temperature (295 K) in order to prevent magnetisation transfer between conformers from disguising true NOE enhancements. Also, in the case of compound **4**, the two α-protons (H-3 and H-6) resonate at very similar chemical shifts, making their selective irradiation difficult. Only the structure of the major conformer of compounds **4** and **5** was investigated. For both compounds, the magnitude of the H-5–H-6 coupling constant (12.3 and 11.0 Hz, respectively) indicated that these protons were approximately *trans* to one another. This deduction was supported by the absence of a large NOE between H-4 and H-5 for both diastereoisomeric compounds. Irradiation of H-6 of compound **4** showed an enhancement at H-3, a result that is only compatable with a *cis* amide bond as shown in Fig. 1. The reverse enhancement of H-6 when H-3 was irradiated was not observed due to the close proximity of these two protons. For compound **5**, no NOE enhancement was observed between protons H-3 and H-6, however an enhancement was observed between H-3 and H-5, a result that is only compatable with a *trans* amide bond as shown in Fig. 2.

These results indicated that the adoption of a *cis* or *trans* amide bond in these eight-membered ring compounds is determined by the relative stereochemistry of the two chiral centres. Molecular mechanics calculations[8] were also consistent with this hypothesis, as they showed that the conformations suggested by NMR allow the methoxycarbonyl substituent at C-6 to adopt a pseudo equatorial position. Structures

‡ All new compounds gave satisfactory spectral data.

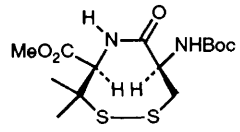


Fig. 1

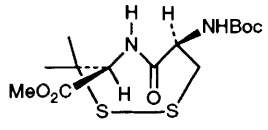


Fig. 2

with the opposite amide bond geometry however place the methoxycarbonyl substituent in a sterically hindered pseudo axial position. However, in view of the very close chemical shift of H-3 and H-6 in compound **4**, on which these deductions rest it was felt prudent to prepare an alternative series of compounds. Thus, compounds **4** and **5** were converted into the *N*-trifluoroacetyl derivatives **8** and **9** respectively by sequential treatment with trifluoroacetic acid, and trifluoroacetic anhydride as shown in Scheme 1. Compared with the *N*-Boc compounds, the NMR spectra of the *N*-trifluoroacetyl derivatives differ in two ways; the intensity of the peaks corresponding to the minor conformers are much reduced in the case of compound **8** and not observed for compound **9**, and the chemical shifts of the α-protons H-3 and H-6 are well separated. The results of a series of low temperature NOE experiments on compounds **8** and **9** were entirely consistent with those of peptides **4** and **5**, peptide **8** having a *cis* amide bond whilst compound **9** has a *trans* amide bond.

In summary, it has been shown that the amide bond geometry of conformationally constrained peptides containing an eight-membered ring disulfide is determined by the relative stereochemistry of the chiral centres.

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