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The Synthesis and Conformational Analysis of a Pair of Diastereomeric, Conformationally Constrained Peptides with Opposite Amide Bond Geometries

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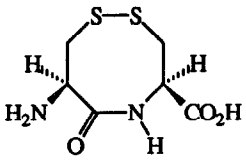
Abstract: *Eight membered ring containing, conformationally constrained peptides derived from cyclo-[(R)-cysteinyl-(R)-penicillamine] contain a cis amide bond, whilst the diastereomeric peptides derived from cyclo-[(R)-cysteinyl-(S)-penicillamine] contain a trans amide bond.*

The use of intramolecular disulphides to constrain linear peptides into biologically active conformations is a widely used technique in medicinal chemistry[2], largely due to the ease with which disulphides can be introduced by the oxidation of cysteine or penicillamine thiols[3]. Despite this, no systematic survey of the conformational effect of introducing a disulphide constraint into a peptide has been conducted. Such a survey should eventually allow the conformation of a disulphide containing peptide to be predicted given the size of the ring formed, the nature and configuration of the thiol containing amino acids, and the nature of the intervening amino acids.

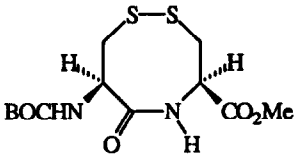
The simplest disulphide containing peptides are those which contain just two amino acids, and so possess an eight membered ring as shown by structures (1) to (4). A number of compounds of this type have been previously described in the literature, thus *cyclo*-[(R)-cysteinyl-(R)-cysteine] (1) and the protected derivative (2) were found by X-ray crystallography to adopt a conformation with a *cis* amide bond[4]. The conformation of compound (1) has been investigated in D_2O, and it was shown that the observed coupling constants were consistent with the conformation determined by X-ray crystallography[5]. Similarly, we have previously shown that derivative (3) exists in chloroform solution as two conformations which interconvert slowly on the nmr timescale[6]. Both conformations of compound (3) were found to have a *cis* amide bond, and they differ in the helicity of the disulphide, with one of the two conformations having an identical ring structure to that determined for compounds (1) and (2) by X-ray crystallography. A *cis* amide bond was also predicted for this compound on the basis molecular mechanics results[7]. However, when compound (1) was incorporated into a heptapeptide, two conformations were observed by nmr in which the amide bond was assigned as *cis* in the major conformer, and *trans* in the minor[8]. Incorporation of compound (1) in to a cyclic hexapeptide analogue of somatostatin, also resulted in the formation of two conformers, however both were determined to possess a *cis* amide bond[9].

By contrast, *N*-phenylacetyl-*cyclo*-[(R)-cysteinyl-(S)-penicillamine] methyl ester (4), was found by X-ray crystallography and nmr techniques to possess a *trans* amide bond both in the solid state and in solution[10].

Hence, an amide bond within an eight membered ring disulphide can adopt either a *cis* or *trans* geometry depending upon the functionality present elsewhere in the molecule. Inspection of molecular models, showed that for disulphide containing rings with less than eight atoms in them, the amide bond could only be accommodated in a *cis* configuration, whilst for nine membered rings and larger, a *trans* amide could be accommodated and would be expected to be preferred for steric reasons. The changeover occurs with eight membered ring disulphides, thus by studying such compounds it should be possible to determine the factors that affect amide bond geometry. In this manuscript, we describe the synthesis and solution conformation of a number of derivatives of compound (4), which have allowed the factor determining the amide bond geometry in these compounds to be determined[11].



(1)



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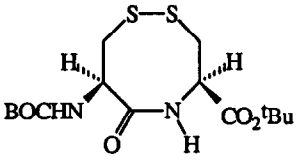

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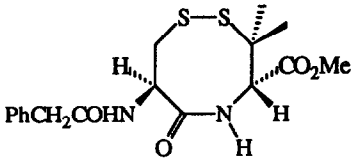


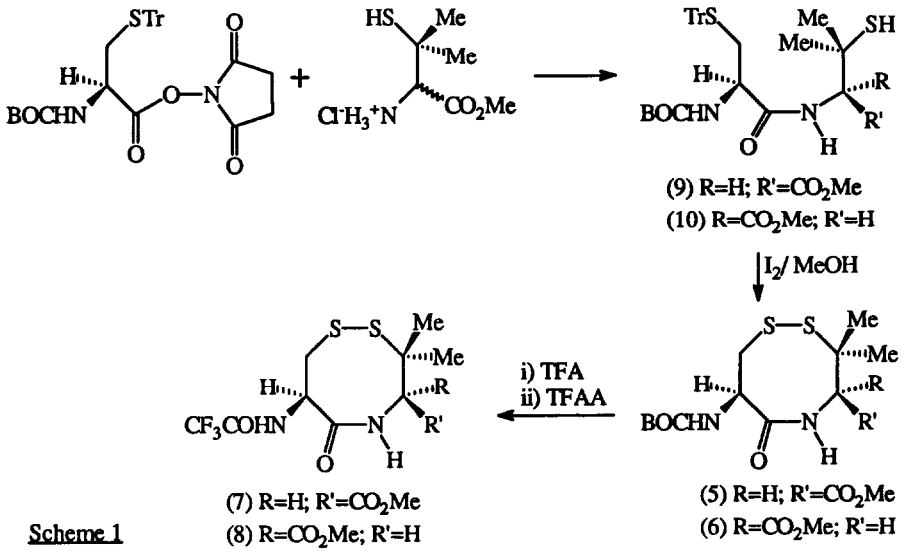
(4)

Comparison of structures (2) and (4), which are known to have different amide bond geometries both in the solid state and in solution, suggested that one or more of three factors may be responsible for this difference; namely the different amine protecting group, the presence of two methyl groups on the *C*-terminal amino acid of compound (4), or the differing absolute configurations of the *C*-terminal amino acids of compounds (2) and (4). Of these, it seemed that the latter two possibilities were most likely, thus the synthesis of compounds (5) and (6), and (7) and (8), in which each pair differs only in the absolute configuration of the penicillamine residue was undertaken.

Synthesis of Compounds (5)-(8)

The synthesis of compounds (5) and (6) was accomplished as shown in Scheme 1. Thus coupling of the *N*-hydroxysuccinimide ester of *N*-Boc-*S*-trityl-(R)-cysteine[12] with (R)- or (S)-penicillamine methyl ester hydrochloride[13] respectively gave the linear dipeptides (9) and (10). It is notable that it was not necessary to protect the thiol function of the penicillamine residue during this coupling in contrast to the situation previously observed with compound (3) in which both thiols had to be protected[5]. Presumably the two methyl groups adjacent to the thiol sterically reduce its nucleophilicity to such an extent that it does not interfere with the peptide coupling. Cyclisation to the desired disulphides (5) and (6) was achieved by treatment of peptides (9) and (10) with iodine in methanol under high dilution conditions[14].

Compounds (7) and (8) which possess an amide rather than a urethane group on the nitrogen atom were prepared from compounds (5) and (6) respectively. Thus treatment of the BOC peptide derivative (5) or (6) with trifluoroacetic acid, followed by treatment of the crude deprotected peptide with trifluoroacetic anhydride gave compounds (7) and (8).



Scheme 1

Conformational Analysis of Compounds (5) to (8)

The 250MHz NMR spectrum of compound (6) showed the expected resonances which were assigned on the basis of their coupling constants and chemical shifts as shown in Table 1 and Figure 2. The two diastereotopic H9 methyl groups occur as a single resonance (1.46ppm) at both 250MHz, and at 400MHz, whilst the diastereotopic H8 protons occur at 2.92 and 3.30ppm respectively. The ^{13}C nmr signals of the diastereotopic C9-carbons are also very close, differing by just 2.6ppm (23.37 and 26.02ppm respectively). The magnitude of the coupling constant between H4 and H5 (11.1Hz) can be used to determine the dihedral angle between these two protons *via* the Karplus equation which has the general form shown in Equation 1. A number of different values have been suggested for the constants A, B, and C[15,16], however the majority of these do not allow the coupling constant to be as large as 11-12Hz[14]. The constants proposed by Thong *et al.*[15] however give Equation 2 which predicts coupling constants as large as 12.8Hz and is used throughout this manuscript. The observed coupling constant, then suggests a single real solution to the quadratic equation, and a dihedral angle of 157° between H4 and H5. At 400MHz, evidence of a second conformation of compound (6) with a population of c.a. 5% was observed by the appearance of additional peaks corresponding to some of the proton resonances, and by the presence of n.O.e. exchange peaks between major and minor conformer resonances *vide infra*. The minor conformer had a coupling constant of 12.2Hz between H4 and H5, corresponding to a dihedral angle of 166°.

$$^3J_{H4\text{-}H5} = A\cos^2\theta + B\cos\theta + C \quad \text{Equation 1}$$
$$^3J_{H4\text{-}H5} = 9.0\cos^2\theta - 3.5\cos\theta + 0.3 \quad \text{Equation 2}$$
$$^3J_{H7\text{-}H8} = 9.4\cos^2\theta - 1.4\cos\theta + 1.6 \quad \text{Equation 3}$$

Figure 1: Numbering System for Compounds (5) and (6): Compounds (7) and (8) are Numbered Analogously

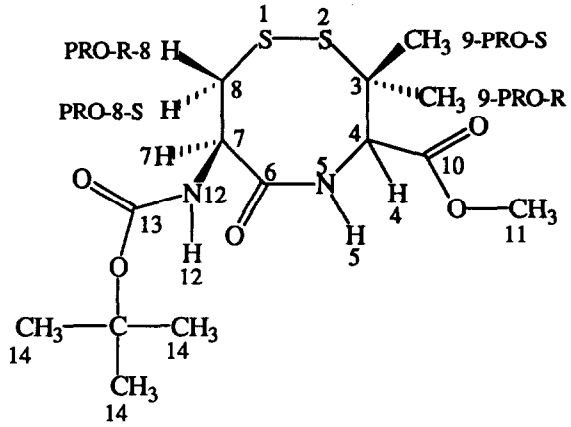


Table 1: Proton Chemical Shifts and Observed n.O.e.'s for the Major Conformer of Compound (6)

Observed n.O.e.	H8 (2.92)	H8 (3.30)	H11	H7	H4	H12	H5
Proton Resonance δ (Assignment)							
1.42 (H14)					s	m	m
1.46 (H9)			s		l		l
2.92 (H8)		l		m		m	m
3.30 (H8)	l			m		s	m
4.26 (H7)		m				m	l
4.93 (H4)						s	s
5.43 (H12)	m			m			
6.48 (H5)				l	m	s	

n.O.e.'s are classified as small (s, less than 4%), medium (m, 4-10%), or large (l, >10%)

Additional conformational information on compound (6) was obtained from a series of n.O.e. experiments in which the major conformer peaks were saturated, the results of these experiments are summarised in Table 1. The enhancements seen upon irradiation of the BOC group protons (H14, 1.42ppm) are not particularly informative, especially as the enhancements to H4 and H5 may be due to partial irradiation of the H9 protons during this experiment. Irradiation of the penicillamine methyl groups (H9, 1.46ppm) shows enhancements to each of the other penicillamine resonances (H4, H5, and H11), but gives no useful conformational information as both methyl groups are being irradiated concomitantly. Irradiation of the cysteine β-protons (H8, 2.92 and 3.30ppm) results in both cases in a large n.O.e. to the other β-proton, and smaller n.O.e.'s to the other cysteine resonances (H7 and H12), and to the amide NH (H5). It is not

possible to assign the stereochemistry of these diastereotopic protons from these n.O.e.'s. Irradiation of the cysteine α-proton (H7, 4.26ppm) however shows an n.O.e. only to the H8 resonance at 3.30ppm, suggesting that this corresponds to the PRO-S cys-β-proton and that the resonance at 2.92ppm must therefore correspond to the PRO-R cys-β-proton. Irradiation of H7 also shows a moderate n.O.e. to H12, and significantly a large n.O.e. to the amide NH (H5, 6.48ppm), a result that is only consistent with the presence of a *trans* amide bond *vide infra*. Irradiation of the penicillamine α-proton shows only small n.O.e.'s to the two NH protons H5 and H12. Irradiation of the BOCNH (H12, 5.43ppm) shows enhancements in the cysteine α-proton and to the cysteine β-proton at 2.92ppm, which has been assigned as corresponding to the PRO-R proton, suggesting that H12 is located on the same face of the molecule as H8-PRO-R proton. Finally, irradiation of the amide NH (H5, 6.48ppm) shows a small enhancement in H12, a moderate enhancement in H4, and significantly a large enhancement in the H7 resonance, mirroring the enhancement seen when H7 is irradiated, and again indicating a *trans* amide bond as discussed in the section on molecular modelling results *vide infra*.

The corresponding nmr data for compound (5) are shown in Table 2. As in the case of compound (6), a second set of peaks corresponding to a minor conformer of compound (5) were observed (population *c.a.* 8%), as were n.O.e. exchange peaks between the two conformers. The two diastereotopic methyl groups (H9) of the major conformer of compound (5) occur at very different chemical shifts (1.18, and 1.44ppm), indicating that they are in different magnetic environments, unlike compound (6) where only a single methyl resonance was observed. The same effect is observed in the ^{13}C nmr spectrum where the two C9 resonances occur 7.0ppm apart (19.63 and 26.65ppm respectively) compared to a separation of just 2.6ppm for compound (6). In the minor conformer, the two methyl groups have much more similar chemical shifts (1.24 and 1.27ppm). The magnitude of the H4-H5 coupling constant for the major conformer of compound (6) (12.3Hz) indicates a dihedral angle of 168° between these two protons, whilst in the minor conformer the coupling constant of 10.0Hz suggests a dihedral angle of 150° between H4 and H5.

Table 2: Proton Chemical Shifts and Observed n.O.e.'s for the Major Conformer of Compound (5)

Observed n.O.e.	H8 (2.88)	H8 (3.01)	H11	H7	H4	H12	H5
Proton Resonance δ (Assignment)							
1.18 (H9)			m		m		l
1.42 (H14)*			m		l		s
1.44 (H9)*			m		l		s
2.88 (H8)				l		m	s
3.01 (H8)				m			
4.63 (H7)		m				m	
4.68 (H4)				m			l
5.84 (H12)	m	s		m			
6.39 (H5)			s		s		

n.O.e.'s are classified as small (s, less than 4%), medium (m, 4-10%), or large (l, >10%); * peaks were irradiated simultaneously

n.O.e. Saturation of the major conformer penicillamine β-methyl resonance at 1.18ppm showed enhancements in the methyl ester, penicillamine α-proton (H4), and a large enhancement in the amide (H5) resonance. When combined with the other n.O.e. experiments *vide infra*, this suggests that this resonance corresponds to the methyl group on the same face of the molecule as the amide NH, and hence that the peak at 1.18ppm corresponds to the PRO-S methyl group. Selective irradiation of the other penicillamine methyl resonance (1.44ppm), and the BOC group (1.42ppm) was not possible due to the small difference in their chemical shifts. However, when they were simultaneously irradiated, enhancements were seen in the resonances corresponding to the amide NH (H5), the methyl ester (H11), and the penicillamine α-proton (H4). It seems likely that these enhancements are due to magnetisation transfer from the penicillamine methyl group rather than from the BOC group, as the latter is at the opposite end of the molecule, and they confirm the diastereomeric assignment of the penicillamine methyl groups. Thus only a small enhancement in the amide (H5) resonance is observed when the methyl group at 1.44ppm is irradiated, but a large enhancement is seen in the α-proton (H4), indicating that this methyl group is on the same face as the penicillamine α-proton and so is the PRO-R methyl group. Irradiation of the H8 protons gave no useful information, as interpretation of the observed n.O.e's is complicated by magnetisation transfer between the two conformers. It was not possible to irradiate H7, without also partially irradiating the H4 resonance, as these two peaks differ by only 0.05ppm in their chemical shift. Irradiation of H7 did however result in the enhancement of only one of the H8 signals (at 3.01ppm), suggesting that this corresponds to the PRO-S H8 proton. Selective irradiation of the H4 resonance was possible, as this signal is a doublet rather then the ddd observed for H7, and a moderate enhancement in the H7 resonance was observed, a result that is only consistent with a *cis* amide bond as indicated by the molecular modelling results *vide infra*. Irradiation of H12 showed the expected n.O.e.'s in all of the cysteine resonances, whilst irradiation of H5 showed small enhancements in the methyl ester, and H4.

The above results when combined with the results of molecular mechanics calculations *vide infra* suggested that the major conformer of methyl *N*-Boc-*cyclo*-[(R)-cysteinyl-(S)-penicillamine] (6) present in $CDCl_3$ solution possesses a *trans* amide bond whilst the major conformer of methyl *N*-Boc-cyclo-[(R)-cysteinyl-(R)-penicillamine] (5) adopts a *cis* amide bond. No information on the amide bond geometry of the minor conformer is available from the above results. However, whilst the assignment of a *trans* amide bond to compound (6) appears unequivocal, the assignment of a *cis* amide bond to compound (5) rests upon an observed n.O.e. between the two α-protons, which have very similar chemical shifts, thus the observed n.O.e. may not be very reliable. Hence in order to confirm the above results, it was felt desirable to prepare an alternative pair of compounds in which the ^1H nmr α-proton resonances were more widely separated. Thus the *N*-trifluoroacetyl compounds (7) and (8) were prepared, as it was anticipated that the strongly electron withdrawing trifluoroacetyl group would shift the cysteine α-proton signal significantly downfield of the penicillamine α-proton resonance. Replacing the BOC group by a trifluoroacetyl group also had the advantage that N12 would be in the form of an amide rather than a urethane, thus conformational results from compounds (7) and (8) might be of more relevance to the conformations adopted by *cyclo*-[cysteinyl-penicillamine] groups within larger peptides.

The observed ^1H nmr chemical shifts and n.O.e's for compound (8) are shown in Table 3, unlike the corresponding *N*-BOC compound (6), only a single set of peaks are observed, suggesting that compound (8) exists as a single conformer in $CDCl_3$. Like compound (6), the two diastereotopic H9 methyl groups of compound (8) are very nearly equivalent (1.48 and 1.49ppm), the corresponding ^{13}C nmr signals for C9, are

again 2.6ppm apart (the same separation occurs in compound (6)), and the H4-H5 coupling constant of 11.2Hz suggests a dihedral angle of 157° between these two protons. The observed n.O.e's for compound (8), are also similar to those observed for compound (6), and in particular a large n.O.e. is observed between H5 and H7, indicating a *trans* amide bond *vide infra*. Thus it appears that the conformation of the eight membered ring in compound (8) is identical to that of the major conformer of compound (6). The observed n.O.e's between the H7 and H8 proton resonances suggest the same diastereotopic assignments of H8 as in compound (6), thus the higher field H8 resonance corresponds to the PRO-R proton, and the lower field resonance to the PRO-S proton.

Table 3: Proton Chemical Shifts and Observed n.O.e.'s for Compound (8)

Observed n.O.e.	H8 (3.02)	H8 (3.41)	H7	H4	H5	H12
Proton Resonance δ (Assignment)						
3.02 (H8)		l	m			
3.40 (H8)	l		s			
4.57 (H7)	s	l			l	s
4.96 (H4)					s	
6.68 (H5)			l	s		
7.31 (H12)	s		s			

n.O.e.'s are classified as small (s, less than 4%), medium (m, 4-10%), or large (l, >10%).

Relevant ^{1}H nmr data for compound (7) are shown in Table 4, gratifyingly the two α-protons (H4 and H7) which in compound (5) were separated by just 0.05ppm are now 0.21ppm apart. Unlike compound (8), the ^{1}H nmr spectrum and n.O.e. spectra of compound (7) showed evidence of two conformations, with the minor conformer having a population of c.a. 4%. As for compound (5), the two diastereotopic H9 methyl groups of the major conformer occur at very different chemical shifts (1.22, and 1.47ppm) as do the corresponding ^{13}C nmr resonances (19.59 and 26.66ppm; a separation of 7.1ppm compared to 7.0ppm for the same peaks in compound (5)), whilst in the minor conformer ^{1}H nmr H9 signals are nearly coincident (1.49, and 1.50ppm). The magnitude of the H4-H5 coupling constant of the major conformer (12.1Hz) suggests a dihedral angle of 165° between these two protons, whilst for the minor conformer the coupling constant is 9.6Hz, suggesting an angle of 147° between H4 and H5. All of this data is very similar to the results obtained for compound (5), and suggests that the conformation of the eight membered disulphide ring does not change for either conformer on changing the amine protecting group. This was confirmed by a set of n.O.e. experiments in which the major conformer peaks were saturated as shown in Table 4.

Irradiation of the penicillamine β-methyl resonance at 1.22ppm showed only a small enhancement in the penicillamine α-proton (H4), and a moderate enhancement in amide NH (H5). Saturation of the other penicillamine β-methyl resonance (1.47ppm) however, showed a large enhancement in the penicillamine α-proton, and no enhancement in the amide NH (H5). These results suggest that the resonance at 1.22ppm corresponds to the PRO-S methyl group on the same face of the eight membered ring as the amide NH, whilst

the resonance at 1.47ppm corresponds to the PRO-R methyl group on the same face as the penicillamine α-proton, the same diastereotopic assignments as in compound (5). Only one of the cysteine β-proton resonances (H8, 2.93ppm) showed an enhancement in the cysteine α-proton (H7), suggesting that this corresponds to the PRO-S H8 proton, and that the resonance at 3.03ppm must correspond to the PRO-R proton. Saturation of the penicillamine α-proton (H4) resulted in the enhancement of both penicillamine β-methyl groups (H9), the amide NH (H5), and significantly the cysteine α-proton (H7), a result that is only consistent with a *cis* amide bond as discussed in the section on molecular mechanics calculations. Irradiation of the penicillamine α-proton (H7) shows a large enhancement in the cysteine α-proton (H4), providing further evidence for a *cis* amide bond, as well as small enhancements in both cysteine β-protons (H8). Finally, irradiation of the amide NH (H5) showed small enhancements in one of the penicillamine β-methyl groups (H9, 1.22ppm), and the penicillamine α-proton (H4). These results are again consistent with those obtained for compound (5), however as the two α-protons are well separated in compound (7), the observed n.O.e. between them is much more reliable.

Table 4: Proton Chemical Shifts and Observed n.O.e.'s for the Major Conformer of Compound (7)

Observed n.O.e.	H9 (1.22)	H9 (1.47)	H8 (2.93)	H8 (3.03)	H4	H7	H5
Proton Resonance δ (Assignment)							
1.22 (H9)		m			s		m
1.47 (H9)	s				l		
2.93 (H8)				l	m		
3.03 (H8)			m				
4.66 (H4)	s	s				m	m
4.87 (H7)			s	s	l		
6.58 (H5)	s				s		

n.O.e.'s are classified as small (s, less than 4%), medium (m, 4-10%), or large (l, >10%).

Molecular Modelling of Cyclo-[Cysteinyl-Penicillamine] Derivatives

A molecular mechanics study of compounds (5) and (6) was undertaken, both to investigate the conformational preferences of these compounds, and to suggest how the conformations could be distinguished by nmr techniques. Calculations were performed on a Silicon Graphics personal IRIS workstation using the Amber forcefield within the Macromodel-3D program[17]. The modelling protocol was as follows: A randomly drawn structure corresponding to either compound (5) or compound (6) was first minimised *in vacuo* using the TNCG method, and the resulting structure was used as the starting point for a grid search. The disulphide bond was chosen as the ring closure bond, and all dihedral angles within the eight membered ring, as well as the angles defined by atoms 3-4-10-O, 6-7-12-13, and 7-12-13-O were rotated in 60° increments. From the resulting set of conformers, those with disulphide bonds of unrealistic length (<100pm, or >250pm) were automatically discarded, and the remainder were minimised *in vacuo* using the TNCG method until the

energy gradient was <0.1kJmol^{-1} to give a set of possible conformers. The resulting structures were ordered according to their energy, and only structures within 50kJmol^{-1} of the global minimum were retained. Details of the low energy structures produced for compound (5) are given in Table 5, and structures produced for compound (6) are given in Table 6.

For compound (5), the modelling results predicted that structures with a *trans* amide bond should be up to 10.7kJmol^{-1} more stable than structures with a *cis* amide bond. However, examination of the *trans* conformers showed that in all cases the two α-protons (H4 and H7) were too far apart to show an n.O.e. between their nmr resonances. Structures with a *cis* amide bond however always brought the two α-protons into close proximity as shown in Figure 2. Hence, based on the combination of nmr and molecular modelling techniques, it can be concluded that the major conformer of compound (5) present in chloroform solution possesses a *cis* amide bond. The observation of an n.O.e. between the two α-protons of a *cyclo*-[(R)-cysteinyl-(R)-cysteine] unit has also been used previously to identify a *cis* amide bond within eight membered ring disulphides[8,9]. Examination of the various conformers of compound (5) with a *cis* amide bond predicted by molecular mechanics calculations, revealed that they all possessed one of two conformations (A and B) for the eight membered ring (with differing orientations for the BOC and methyl ester groups within each set), these are shown in Figure 2, and differ mainly in the helicity of the disulphide (M-helical disulphide for conformation A, and P-helical disulphide for conformation B).

Table 5: Selected Parameters for the Predicted Low Energy Conformations of Compound (5)

No.	Energy (kJmol^{-1})	Amide Bond Geometry	H4-H5 Dihedral Angle	J_{H7-H8} (*J*-PRO-R H8 first)
1	0	trans	145.6°	11.2, and 5.3Hz
2	4.0	trans	143.9°	11.3, and 5.2Hz
3	10.7	cis	144.8°	11.0, and 5.7Hz
4	12.5	cis	144.2°	11.9, and 5.7Hz
5	12.9	trans	146.7°	10.6, and 6.1Hz
6	13.6	cis	144.7°	11.0, and 5.6Hz
7	14.6	trans	144.6°	10.7, and 6.0Hz
8	15.0	trans	144.4°	10.7, and 5.9Hz
9	15.4	cis	144.1°	11.0, and 5.6Hz
10	15.7	trans	148.9°	10.8, and 5.0Hz
11	15.8	trans	146.8°	10.6, and 6.1Hz
12	16.0	trans	151.5°	11.0, and 5.6Hz
13	17.2	trans	154.6°	11.1, and 5.6Hz
14	17.4	trans	147.1°	10.8, and 5.8Hz
15	17.4	cis	163.1°	11.6, and 2.1Hz
16	17.6	trans	144.6°	10.7, and 6.0Hz
17	18.6	cis	164.5°	11.6, and 2.1Hz
18	19.4	cis	143.9°	11.4, and 5.1Hz

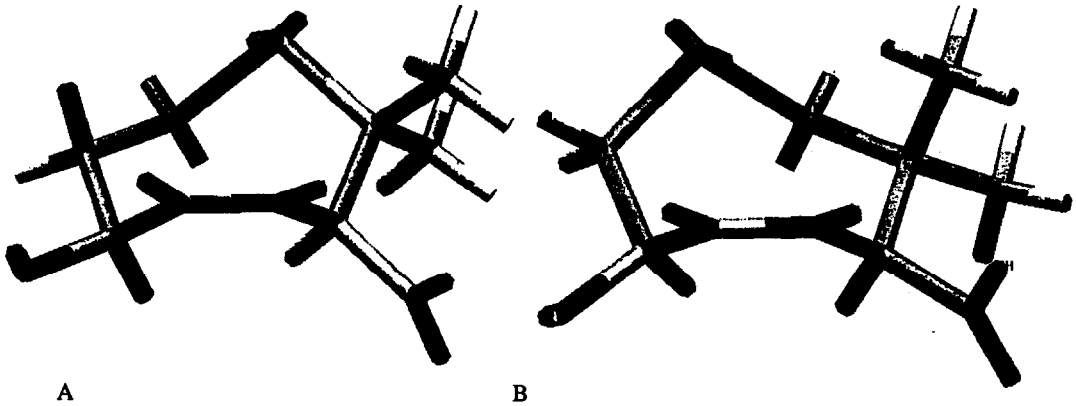
The energies have been adjusted to give conformer No. 1 an energy of 0, *J* values are calculated within the Macromodel program

Table 6: Selected Parameters for the Predicted Low Energy Conformations of Compound (6)

No.	Energy (KJmol^{-1})	Amide Bond Geometry	H4-H5 Dihedral Angle	J_{H7-H8} (*J*-PRO-R H8 first)
1	0	cis	16.5°	11.3, and 5.2Hz
2	2.7	cis	16.0°	11.4, and 5.1Hz
3	6.7	cis	168.8°	9.6, and 5.5Hz
4	9.2	trans	20.6°	11.1, and 5.5Hz
5	10.2	cis	168.7°	9.6, and 5.7Hz
6	11.5	cis	14.3°	11.4, and 5.0Hz
7	12.3	cis	169.8°	9.7, and 5.2Hz
8	12.3	trans	179.2°	11.8, and 3.6Hz
9	12.6	cis	171.6°	9.7, and 5.1Hz
10	13.3	trans	176.3°	11.7, and 4.0Hz
11	13.7	trans	175.3°	11.7, and 3.9Hz
12	14.7	cis	14.1°	11.4, and 5.0Hz
13	14.8	cis	168.2°	9.4, and 6.3Hz
14	14.9	trans	57.3°	3.4, and 3.1Hz
15	15.0	cis	164.5°	4.5, and 2.3Hz
16	15.3	trans	56.3°	3.4, and 3.1Hz
17	15.6	trans	55.8°	3.5, and 3.0Hz
18	15.9	cis	169.5°	9.4, and 6.2Hz
19	15.9	cis	177.1°	9.6, and 5.5Hz
20	16.0	trans	176.9°	11.7, and 3.9Hz
21	16.5	trans	175.2°	11.7, and 3.8Hz
22	16.8	trans	22.7°	11.3, and 5.2Hz
23	17.9	cis	165.1°	4.5, and 2.3Hz
24	19.0	cis	168.5°	9.4, and 6.2Hz
25	19.3	trans	173.5°	11.8, and 3.3Hz
26	19.4	cis	167.3°	4.5, and 2.3Hz
27	19.6	cis	165.0°	4.5, and 2.3Hz
28	19.9	cis	177.0°	9.6, and 5.6Hz
29	20.3	cis	169.7°	9.4, and 6.2Hz
30	20.3	cis	15.4°	11.7, and 4.5Hz
31	20.4	trans	150.6°	5.6, and 1.6Hz
32	21.1	trans	62.3°	3.2, and 3.3Hz
33	21.1	trans	18.0°	11.1, and 5.6Hz
34	21.3	trans	22.5°	10.5, and 6.3Hz
35	21.7	trans	14.5°	11.2, and 5.4Hz
36	21.8	trans	148.7°	5.5, and 1.6Hz

The energies have been adjusted to give conformer No. 1 an energy of 0, *J* values are calculated within the Macromodel program

Figure 2: Conformations A and B of Compound (5) with a Cis Amide Bond.



The BOC and methyl ester groups are omitted for clarity. Conformer A is structure 3 found by molecular mechanics calculations, whilst structure B is conformer 15 shown in Table 5.

The two eight membered ring conformations of compound (5) with a *cis* amide bond can be distinguished both by the H4-H5 dihedral angle (144° for structure A, and 164° for structure B), and by their H7-H8 coupling constants, using Equation 3 derived by Kopple *et al.*[18]. In both cases the H7-$H8_{PRO-R}$ coupling constant is predicted to be 11-12Hz, whilst the H7-$H8_{PRO-S}$ coupling constant is predicted to be 5-6Hz for conformer A, but 2.1Hz for conformer B. The observed H7-H8 coupling constants for the major conformer of compound (5) are 10.8 and 2.7Hz, values consistent with those required for a conformation with a *cis* amide bond and a P helical disulphide as shown in structure B of Figure 2. The H4-H5 coupling constant of 11.1Hz, for the major conformer of compound (5) in $CDCl_3$ corresponds to a dihedral angle of 157°, again more consistent with the value required for a conformation of type B than type A.

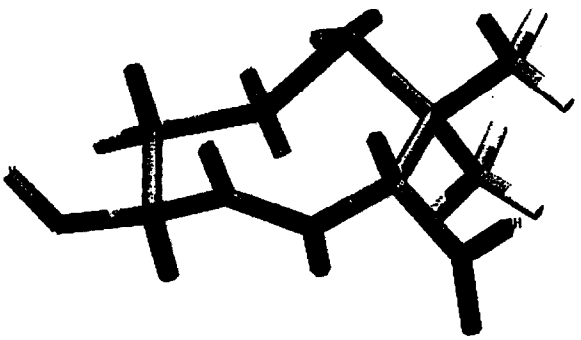
The nmr data for the major conformer of the *N*-trifluoroacetamide compound (7), are also consistent with a structure of type B found by molecular mechanics calculations. Thus the H4-H5 coupling constant of 11.2Hz suggests a dihedral angle of 157°, and the H7-H8 coupling constants of 10.8, and 2.8Hz, are all consistent with the values predicted for structure B.

In the case of compound (6), the results of the molecular mechanics calculations shown in Table 6 suggest that structures with a *cis* amide bond should be at least 9.2kJmol^{-1} more stable than those with a *trans* amide bond. However, none of the *cis* structures bring H5 and H7 into close proximity as indicated by the observed n.O.e. between these two protons. These protons are brought close together by structures with a *trans* amide bond as shown in Figure 3. Indeed the observation of an n.O.e. between an amide NH and the successive α-CH is well known to be characteristic of peptides and proteins with *trans* amide bonds, and can be used to sequence peptides[19]. Hence it can be concluded, that the major conformer of compound (6) observed in $CDCl_3$ possesses a *trans* amide bond. Comparing the various structures in Table 6 with a *trans* amide bond, it is apparent that there is considerably more variation than was found for compound (5), with structures with various H4-H5 dihedral angles being predicted. However, the nmr data for compound (6) suggests that the H4-H5 dihedral angle must be >140°, and only two types of ring conformation fit this requirement. The first of these corresponds to structures such as number 8 in Table 6, and is shown as

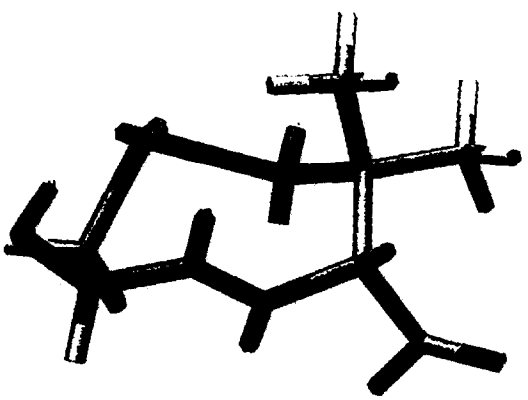
conformation A in Figure 3. The other ring conformation is represented by structures 31 and 36 in Table 6, and is shown as conformation B in Figure 3. Conformations A and B differ mainly in the helicity of the disulphide (P in conformer A and M in conformer B), and the various structures given in Table 6 which correspond to one of these conformations differ in the orientation of the BOC and methyl ester groups. The nmr data for the major conformer of compound (6) present in $CDCl_3$ solution, are more compatible with those required for a structure of type A than B. Thus the observed H7-H8 coupling constants of 5.2 and 11.0Hz are consistent with the values of 3.6-4.0 and 11.8Hz required for structure A, but not with the values of 1.6 and 5.6Hz required for structure B. The magnitude of the H4-H5 coupling constant (11.1Hz) however suggests a dihedral angle of 157° between these two protons which would appear to be more consistent with a structure of type B (150°), than with type A (173-179°). However, the H4-H5 coupling constant was calculated using Equation 2[16] in which the maximum possible coupling constant is 12.8Hz (at 180°), whereas all of the other equations that have been proposed for calculating this coupling constant[15] have much lower maximum values, so that a coupling constant of 11.1Hz would correspond to a dihedral angle much larger than 157°. Also the nature of the curve corresponding to Equation 2 is such that in the region around a dihedral angle of 180°, a small error in the coupling constant will result in a relatively large error in the dihedral angle.

Figure 3: Conformations A and B of Compound (6) with a Trans Amide Bond.

A



B



The BOC and methyl ester groups are omitted for clarity. Conformer A is structure 8 found by molecular mechanics calculations, whilst structure B is conformer 31 shown in Table 6.

The *N*-trifluoroacetamide derivative (8) exists as a single conformation in $CDCl_3$, and again this is consistent with a structure of type A with a *trans* amide bond and a P helical disulphide. Thus the H7-H8 coupling constants are 5.1 and 11.1Hz, almost identical with the values obtained for compound (6), and the H4-H5 coupling constant of 11.2Hz again gives a dihedral angle of 157°.

The structure of the minor conformer of compound (5), cannot be so fully determined, as most of the nmr signals are either broadened or obscured by other peaks in the spectrum. However, the H4-H5 coupling constant of 12.1Hz, gives a H4-H5 dihedral angle of 166°. The two penicillamine β-methyl groups have very similar proton chemical shifts in the minor conformer, which is in contrast to the major conformer of compound (5) but very similar to the situation observed for the major conformer of compound (6) where these two peaks are coincident. The same effect is observed for the minor conformer of compound (7), and this suggests that the minor conformers of compounds (5) and (7) may possess a *trans* amide bond rather than *cis* amide observed for the major conformer. Not enough peaks are visible in the ^1H nmr spectrum of the minor conformer of compound (6) to allow any conformational information to be obtained.

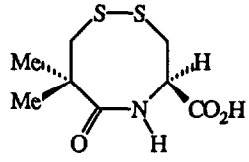
The molecular modelling results also provide an explanation for the differing amide bond geometries of compounds (5,7) and (6,8). As can be seen from Figures 2 and 3, the preferred conformations allow the amine and carboxylic acid groups to adopt unhindered *pseudo* equatorial positions, whilst the other amide bond geometry would force one of the groups to adopt a relatively hindered *pseudo* axial position. Thus a *trans* amide in compound (5) or (7) would bring the carboxylic acid group into the position adopted by H4 in Figure 3, which is in close proximity to the amide carbonyl. Similarly, a *cis* amide in compound (6) or (8) would bring the carboxylic acid group into the position adopted by H4 in Figure 2, and would result in a severe steric interaction with H7.

Comparison with known X-Ray Structures

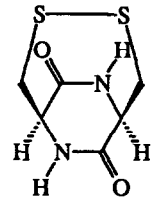
The solution conformations of compound (5) and (7) can be compared with the previously published crystal structures of the related compounds (1) and (2), which possess the same stereochemistry at each chiral center[4]. Similarly, the conformations of compounds (6) and (8) can be compared with the crystal structure of compound (4) which differs only in the amine protecting group[10]. The structure of the major conformer of compounds (5) and (7) present in $CDCl_3$ solution is identical to the solid state structure of compounds (1) and (2), in particular all the structures possess a *cis* amide bond, and a right handed P-helical disulphide. Similarly, the solution conformation of compounds (6) and (8) appears identical to the solid state structure of compound (4), in that all three structures possess a *trans* amide bond, and a P-helical disulphide.

Two other relevant crystal structures have been reported, those of compounds (11)[20] and (12)[21]. Compound (11) which possesses only one chiral centre within an eight membered ring containing a disulphide and amide bond was found to form crystals with two distinct molecular conformations present. Both conformations of possess a *trans* amide bond, and have the Cys-α-proton and NH approximately *trans* to one another, but they differ in the helicity of the disulphide. Compound (12) was found to crystalise in a conformation with an M-helical disulphide, and in this case the Cys-α-protons and NH's are forced to be *cis* to one another due to the presence of the diketopiperazine ring. In solution, compound (12) was shown by CD and ^{13}C nmr measurements to exist as a mixture of two conformations differing in the disulphide helicity[22]. These are the only reported cases of an M-helical conformation being found in the solid state structure of this

type of compound, though there have been previous reports of M-helical conformations being formed as minor conformations in solution[6,9].



(11)



(12)

Conclusions

Methyl *N*-Boc-*cyclo*-[(R)-cysteinyl-(R)-penicillamine] (5) and methyl *N*-trifluoroacetyl-*cyclo*-[(R)-cysteinyl-(R)-penicillamine] (7) exist in chloroform solution as a mixture of two slowly interconverting conformers. The major conformer has a *cis* amide bond within the eight membered ring and a P-helical disulphide, giving a ring conformation that is identical to that previously found for *cyclo*-[(R)-cysteinyl-(R)-cysteine] and its derivatives[4]. The structure of the minor conformer of compounds (5) and (7) is less certain, though based on the ^{1}H nmr chemical shifts it may have a *trans* amide bond.

Methyl *N*-Boc-*cyclo*-[(R)-cysteinyl-(S)-penicillamine] (6) exists as two interconverting conformers in chloroform solution, but only a single conformation was detected for methyl *N*-trifluoroacetyl-*cyclo*-[(R)-cysteinyl-(S)-penicillamine] (8). The structure of the major conformer of compound (6), and of compound (8) contains a *trans* amide bond and a P-helical disulphide, giving a ring conformation identical to that previously reported for the crystal structure of the *N*-phenylacetyl derivative (4)[10]. No information on the structure of the minor conformer of compound (6) could be obtained.

Molecular mechanics calculations were found to be able to produce a set of minimum energy conformations which included the experimentally observed structures for compounds (5) to (8). However, the calculations were unable to calculate the correct relative energies of the various conformations, both in terms of the amide bond geometry, and the disulphide helicity.

The difference in the amide bond geometry of compounds (5/7) and (6/8) is postulated to be due to the amine and carboxylic acid groups adopting the least hindered sites on the eight membered ring. For compounds (5) and (7), this is only possible if the amide bond adopts a *cis* configuration, whilst for compounds (6) and (8), a *trans* amide bond is required.

Further work on these and related compounds is currently underway, and will be reported in due course. In particular, the use of compounds (5) to (8) as building blocks for conformationally constrained peptides containing *cis* or *trans* amide bonds at particular sites is being investigated, as is the conformation of related compounds.

Experimental

^{1}H NMR spectra were recorded at 250MHz on a Brucker AM250 spectrometer fitted with a ^{1}H-^{13}C dual probe, or at 400MHz on a Brucker WH400 spectrometer, and were recorded at 293K in $CDCl_3$ unless otherwise stated. Spectra were internally referenced either to TMS or residual $CHCl_3$, and peaks are reported

in ppm downfield of TMS. Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), some combination of these, broad (br), or multiplet (m). ^{13}C NMR spectra were recorded at 62.5MHz or 100MHz on the same spectrometers as ^{1}H NMR spectra, at 293K and in $CDCl_3$ unless otherwise stated. Spectra were referenced to the $CDCl_3$ peak, and are reported in ppm downfield of TMS. Peak assignments were made by DEPT editing of the spectra, and a * indicates that peak assignments may be interchanged. Infra red spectra were recorded on a Perkin Elmer 1600 series FTIR spectrometer, only characteristic absorptions are reported, and peaks are reported as strong (s), moderate (m), weak (w), or broad (br). Mass spectra were recorded using the FAB technique (Cs^+ ion bombardment at 25kV) on a VG Autospec spectrometer, or by chemical ionisation (CI) with ammonia on a VG model 12-253 quadrupole spectrometer. Only significant fragment ions are reported, and only molecular ions are assigned. Optical rotations were recorded on a Perkin Elmer 141 polarimeter, and are reported along with the solvent and concentration in g/100ml. Melting points are uncorrected. Elemental analyses were performed on a Carlo Erba Model 1106 analyser.

Flash chromatography[23] was carried out on 40-60μm mesh silica, thin layer chromatography was carried out on aluminium backed silica plates (0.25mm depth of silica containing UV254), and the plates were visualised with u.v. light, and/or dodecamolybdic acid as appropriate.

Methyl N-(N-Boc-S-Trityl-(R)-Cysteinyl)-(S)-Penicillamine (10)

(S)-Penicillamine methyl ester hydrochloride[13] (2.0g, 10mMol) was dissolved in CH_2Cl_2 (200ml), triethylamine (1.5ml, 10.4mMol) was added, and the resulting solution stirred at RT for 5 minutes. *N*-Boc-*S*-trityl-(R)-cysteine-*N*-hydroxysuccinimide ester[12] (6.0g, 10.7mMol) was added and the solution stirred at RT for 18 hours. The solution was washed successively with dilute hydrochloric acid (2x100ml), water (100ml), dilute aqueous sodium hydrogen carbonate solution (2x100ml), and water (100ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by flash chromatography (gradient of $CHCl_3$ to 5% EtOAc/$CHCl_3$), giving methyl *N*-(*N*-Boc-*S*-trityl-(R)-cysteinyl)-(S)-penicillamine (10) (1.6g; 26%) as a white crystalline solid. m.p. 66-68°C; $[\alpha]_D^{26}$ +48.3° (c. 2.0 $CHCl_3$); υ_{max} ($CHCl_3$) 3423 m, 3328 m, 1741 s, 1680 s, and 732cm^{-1}; δ_H 1.29 (3H, s, CH_3CS), 1.43 (12H, s, $OC(CH_3)_3$ + CH_3CS), 1.94 (1H, s, SH), 2.5-2.8 (2H, m, CH_2S), 3.66 (3H, s, OCH_3), 3.8-4.0 (1H, br, cys-α-CH), 4.57 (1H, d *J* 10.0Hz, pen-α-CH), 4.98 (1H, d *J* 7.8Hz, BOCHN), 7.15 (1H, d *J* 10.1Hz, CONH), 7.1-7.5 (15H, m, ArH); δ_C 28.40 ($OC(\underline{C}H_3)_3$), 29.17 ($\underline{C}H_3CS$), 30.67 ($\underline{C}H_3CS$), 46.42 (CH_2S), 52.01 (NCH), 53.63 (NCH), 60.24 ($Me_2\underline{C}S$), 67.11 (OCH_3), 77.38 (CPh_3), 80.18 ($OCMe_3$), 126.84 (ArCH), 128.00 (ArCH), 129.41 (ArCH), 144.25 (ArC), 155.14 (NCO_2), 170.17* (CO_2), 170.24* (CON); m/z (FAB) 1215 (disulphide$^+$), 631 (thiol+Na$^+$).

Methyl N-(N-Boc-S-Trityl-(R)-Cysteinyl)-(R)-Penicillamine (9)

N-Boc-*S*-trityl-(R)-cysteine-*N*-hydroxysuccinimide ester[12] (5.2g, 9.2mMol) and (R)-penicillamine methyl ester hydrochloride[13] (2.1g, 13mMol) were dissolved in CH_2Cl_2 (100ml), and triethylamine (1.4g, 13mMol) was added. The reaction mixture was stirred at RT for 48 hours, filtered, and the filtrate washed with 5% aqueous citric acid (2x100ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by flash chromatography (gradient of CH_2Cl_2 to 5% Et_2O/CH_2Cl_2), giving methyl *N*-(*N*-Boc-*S*-trityl-(R)-cysteinyl)-(R)-penicillamine (9) (2.4g; 43%) as a pale yellow foam. m.p. 85-86°C; $[\alpha]_D^{23}$ -5.2° (c. 0.125 $CHCl_3$); υ_{max} ($CHCl_3$) 3684 w, 3616 w, 3427 br, 1716 s, and 1217cm^{-1} s; δ_H 1.42 (3H, s, CH_3CS), 1.44 (3H,

s, CH_3CS), 1.47 (9H, s, $OC(CH_3)_3$), 2.7-3.1 (2H, m, CH_2S), 3.83 (3H, s, OCH_3), 4.6-4.7 (2H, m, 2xα-CH), 5.91 (1H, d *J* 6.7Hz, BOCHN), 6.55 (1H, d *J* 9.2Hz, CONH), 7.1-7.5 (15H, m, ArH); δ_C 23.40 ($\underline{C}H_3CS$), 24.95 ($\underline{C}H_3CS$), 28.31 ($OC(\underline{C}H_3)_3$), 37.86 (CH_2S), 52.44 (NCH), 53.27 (NCH), 62.39 (Me_2CS), 63.73 (OCH_3), 80.75 ($OCMe_3$), 127.27 (ArCH), 128.75 (ArCH), 129.12 (ArCH), 142.32 (ArC), 156.28 (NCO_2), 167.51* (CO_2), 169.83* (CON); m/z (FAB) 1238 (disulphide+Na^+), 1216 (disulphide$^+$), 892, 663, 631 (thiol+Na^+), 369.

Methyl N-Boc-cyclo-[(R)-Cysteinyl-(S)-Penicillamine] (6)

Dipeptide (10) (1.5g, 2.5mMol) was dissolved in MeOH (30ml) and CH_2Cl_2 (20ml) and added to a solution of iodine (1.8g, 7.1mMol) in MeOH (1800ml) over a period of 5 hours *via* a syringe pump. The resulting solution was then stirred at RT for 18hours, following which excess iodine was destroyed by addition of aqueous sodium thiosulphate solution, and the mixture was concentrated *in vacuo*. The residue was taken up into a mixture of ethyl acetate (100ml) and aqueous 5% citric acid (100ml), and the separated organic phase was washed successively with dilute hydrochloric acid (100ml), dilute aqueous sodium hydrogen carbonate (2x100ml), and water (100ml). The ethyl acetate solution was dried ($MgSO_4$), and evaporated *in vacuo*. to yield an oil which was purified by flash chromatography (gradient of $CHCl_3$ to 5% EtOAc/$CHCl_3$), giving methyl *N*-Boc-*cyclo*-[(**R**)-cysteinyl-(**S**)-penicillamine] (6) (200mg; 22%) as an amorphous white solid. m.p. 138-139°C; (Found: C, 46.4; H, 6.6. $C_{14}H_{24}N_2O_5S_2$ requires: C, 46.1; H6.6%); $[\alpha]_D^{28}$ +99.6° (c. 0.5 $CHCl_3$); υ_{max} ($CHCl_3$) 3423 m, 1742 s, 1703 s, and 1668cm^{-1} s; δ_H (major conformer) 1.42 (9H, s, $OC(CH_3)_3$), 1.46 (6H, s, $(CH_3)_2CS$), 2.92 (1H, dd *J* 14.0, 11.2Hz, CH_2S), 3.30 (1H, dd, *J* 14.1, 5.0Hz, CH_2S), 3.74 (3H, s, OCH_3), 4.26 (1H, ddd *J* 11.7, 7.5, 5.0Hz, cys-α-CH), 4.93 (1H, d *J* 11.2Hz, pen-α-CH), 5.43 (1H, d *J* 7.4Hz, BOCHN), 6.48 (1H, d *J* 11.0Hz, CONH); (minor conformer) 1.44 (9H, s, $OC(CH_3)_3$), 3.80 (3H, s, OCH_3), 4.0-4.1 (1H, m, cys-α-CH), 4.68 (1H, d *J* 12.2Hz, pen-α-CH), 5.82 (1H, br, BOCHN), peaks corresponding to the other protons in the minor conformer were not observed; δ_C 23.37 ($\underline{C}H_3CS$), 26.02 ($\underline{C}H_3CS$), 28.27 ($OC(\underline{C}H_3)_3$), 46.57 (CH_2S), 52.48 (NCH), 54.30 (NCH), 57.12 (Me_2CS), 63.71 (OCH_3), 80.29 (Me_3CO), 154.81 (NCO_2), 167.25* (CON), 172.84* (CO_2); m/z (FAB) 387 (M^++Na), 365 (MH^+), 309, 265, 243, 130.

Methyl N-Boc-cyclo-[(R)-Cysteinyl-(R)-Penicillamine] (5)

Dipeptide (9) (2.4g, 4.6mMol) was dissolved in methanol (50ml) and added to a solution of iodine (3.5g, 14mMol) in MeOH (1000ml) over a period of 24 hours *via* a syringe pump. Aqueous sodium thiosulphate solution (200ml, 0.1M) was added to the reaction mixture to destroy excess iodine, following which the solution was concentrated *in vacuo* to c.a. 200ml. The residue was extracted with ethyl acetate (100ml), and the organic solution washed successively with dilute hydrochloric acid (100ml), and dilute aqueous sodium carbonate (100ml). The organic solution was dried ($MgSO_4$), and evaporated *in vacuo* to yield the crude product which was purified by flash chromatography ($CHCl_3$), giving methyl *N*-Boc-*cyclo*-[(**R**)-cysteinyl-(**R**)-penicillamine] (5) (350mg; 21%) as a white solid. m.p. 139-140°C; (Found: C, 46.3; H, 6.65. $C_{14}H_{24}N_2O_5S_2$ requires: C, 46.1; H6.6%); $[\alpha]_D^{28}$ +19.8° (c. 0.5 $CHCl_3$); υ_{max} ($CHCl_3$) 3617 w, 3423 m, 1741 s, 1701 s, 1669 s, and 1216cm^{-1} s; δ_H (major conformer) 1.18 (3H, s, CH_3CS), 1.42 (9H, s, $OC(CH_3)_3$), 1.44 (3H, s, CH_3CS), 2.88 (1H, dd *J* 14.3, 10.8Hz, CH_2S), 3.01 (1H, dd *J* 14.3, 2.7Hz, CH_2S), 3.80 (3H, s, OCH_3), 4.63 (1H, ddd *J* 10.2, 6.8, 2.6Hz, cys-α-CH), 4.68 (1H, d *J* 12.1Hz, pen-α-CH), 5.84

(1H, d *J* 6.8Hz, BOCHN), 6.39 (1H, d *J* 12.3Hz, CONH); (minor conformer) 1.24 (3H, s, CH_3CS), 1.27 (3H, s, CH_3CS), 1.43 (9H, s, $OC(CH_3)_3$), 2.63 (1H, t *J* 11.0Hz, CH_2S), 3.3-3.4 (1H, m, CH_2S), 3.78 (3H, s, OCH_3), 4.81 (1H, d *J* 10.0Hz, pen-α-CH), 5.10 (1H, br, cys-α-CH), 5.39 (1H, br, BOCHN), 6.33 (1H, d *J* 10.0Hz, CONH); δ_C 19.63 ($\underline{C}H_3CS$), 26.65 ($\underline{C}H_3CS$), 28.16 ($OC(\underline{C}H_3)_3$), 41.86 (CH_2S), 52.54 (NCH), 53.19 (NCH), 56.25 (Me_2CS), 58.94 (OCH_3) 80.04 (Me_3CO), 154.38 (NCO_2), 169.46* (CON), 171.29* (CO_2); m/z (CI) 365 (MH^+), 309, 291, 265.

Methyl N-Trifluoroacetyl-cyclo-[(R)-Cysteinyl-(S)-Penicillamine] (8)

Cyclic dipeptide (6) (0.1g, 0.3mMol) was dissolved in CH_2Cl_2 (10ml), and trifluoroacetic acid (2ml) was added. The resulting solution was stirred at RT for 6 hours, then evaporated *in vacuo*, leaving a thick oil which was redissolved in CH_2Cl_2. Triethylamine (0.1g, 1.1mMol) and trifluoroacetic anhydride (74mg, 0.3mMol) were added, and the solution stirred at RT for 24 hours. The solvent and excess reagents were evaporated *in vacuo*, and the residue subjected to flash chromatography ($CHCl_3$) giving methyl *N*-trifluoroacetyl-cyclo-[(R)-cysteinyl-(S)-penicillamine] (7) (0.01g, 10%) as a white solid. m.p. 176-178°C; $[\alpha]_D^{28}$ +111° (c. 0.4 $CHCl_3$); υ_{max} ($CHCl_3$) 3683 w, 3620 w, 3377 m, 1727 s, 1723 s, 1671 s, and 772cm^{-1} s; δ_H 1.48 (3H, s, CH_3CS), 1.49 (3H, s, CH_3CS), 3.02 (1H, dd *J* 14.1, 11.1Hz, CH_2S), 3.40 (1H, dd *J* 14.1, 5.1Hz, CH_2S), 3.78 (3H, s, OCH_3), 4.57 (1H, ddd *J* 11.7, 7.0. 5.2Hz, cys-α-CH), 4.96 (1H, d *J* 11.2Hz, pen-α-CH), 6.68 (1H, d *J* 11.1Hz, CHCONH), 7.31 (1H, br, CF_3CONH); δ_C 23.26 ($\underline{C}H_3CS$), 25.85 ($\underline{C}H_3CS$), 45.15 (CH_2S), 52.52 (NCH), 52.94 (NCH), 57.34 (Me_2CS), 63.63 (OCH_3), 113.79 (CF_3), 156.32 ($CF_3\underline{C}O$), 168.44* (CON), 169.87* (CO_2); m/z (CI) 378 ($M+NH_4^+$), 361 (MH^+), 263, 210, 174.

Methyl N-Trifluoroacetyl-cyclo-[(R)-Cysteinyl-(R)-Penicillamine] (7)

Cyclic dipeptide (5) (0.1g, 0.3mMol) was dissolved in CH_2Cl_2 (10ml), and trifluoroacetic acid (2ml) was added. The resulting solution was stirred at RT for 6 hours, then evaporated *in vacuo*, leaving a thick oil which was redissolved in CH_2Cl_2. Triethylamine (0.1g, 1.1mMol) and trifluoroacetic anhydride (73mg, 0.3mMol) were added, and the solution stirred at RT for 18 hours. The solvent and excess reagents were evaporated *in vacuo*, and the residue subjected to flash chromatography ($CHCl_3$) giving methyl *N*-trifluoroacetyl-cyclo-[(R)-cysteinyl-(R)-penicillamine] (7) (0.01g, 10%) as a white solid. m.p. 173-174°C; $[\alpha]_D^{28}$ +3.6° (c. 0.5 $CHCl_3$); υ_{max} ($CHCl_3$) 3381 s, 1732 m, and 1673cm^{-1} s; δ_H (major conformer) 1.21 (3H, s, CH_3CS), 1.47 (3H, s, CH_3CS), 2.92 (1H, dd *J* 14.3, 10.8Hz, CH_2S), 3.04 (1H, dd *J* 14.3, 2.8Hz, CH_2S), 3.82 (3H, s, OCH_3), 4.66 (1H, d *J* 12.1Hz, pen-α-CH), 4.87 (1H, ddd *J* 10.1, 6.6. 2.8Hz, cys-α-CH), 6.58 (1H, d *J* 11.9Hz, CHCONH), 7.72 (1H, d *J* 6.1Hz, CF_3CONH), (minor conformer) 1.49 (3H, s, CH_3CS), 1.50 (3H, s, CH_3CS), 2.71 (1H, t *J* 12.0Hz, CH_2S), 3.78 (3H, s, OCH_3), 4.77 (1H, d *J* 9.6Hz, pen-α-CH), 5.3-5.4 (1H, m, cys-α-CH), 6.49 (1H, d *J* 9.5Hz, CONH), peaks corresponding to the other protons in the minor conformer were not observed; δ_C 19.59 ($\underline{C}H_3CS$), 26.66 ($\underline{C}H_3CS$), 40.65 (CH_2S), 52.43 (NCH), 52.75 (NCH), 56.29 (Me_2CS), 59.00 (OCH_3), 116.71 (CF_3), 156.24 ($CF_3\underline{C}O$), 169.05* (CON), 169.43* (CO_2); m/z (CI) 378 ($M+NH_4^+$), 361 (MH^+), 265, 174.

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