Tetrahedron Vol. 49, No. 26, pp. 5891-5904, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00
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The Conformation of *Cyclo*-[(R)-Cysteinyl-(R)-Cysteine] in Solution

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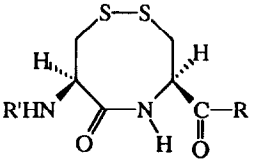
(*Received in UK* 15 *March* 1993)

Key Words: Cyclo[(R)-Cysteinyl-(R)-Cysteine]; Conformation; Molecular Modelling; NMR; Peptide

Abstract: *The conformation in solution of the conformationally constrained cyclic dipeptide N-BOC-[cyclo-(R)-cysteinyl-(R)-cysteine] t-butyl ester is investigated by NMR and molecular mechanics techniques. The compound is found to exist as two slowly interconverting conformations in chloroform, both of which have a cis amide bond.*

The use of disulphides to introduce conformational constraints into biologically active peptides is a well established procedure[1]. However, although a conformational analysis of the resulting peptides is often undertaken, no systematic investigation of the conformations adopted by cyclic disulphide containing peptides has been undertaken. Therefore, we have started a project aimed at the conformational analysis of a variety of cyclic cysteine containing peptides differing in their ring sizes, absolute configurations, and substitution patterns. In this paper the conformation of a derivative of the simplest such compound cyclo-[(R)-cysteinyl-(R)-cysteine] (1) in solution is described.



(1) R=OH, R'=H
(2) R=OMe, R'=BOC
(3) R=LeuGly, R'=LeuArgArg
(4) R=O^{t}Bu, R'=BOC

The conformations of disulphide (1) and its methyl ester derivative (2) have previously been investigated by X-ray crystallography[2], and in both cases the amide bond was found to be *cis*, indeed the conformation of the two eight membered rings in these compounds were found to be superimposable. Compound (1) was also investigated by early molecular mechanics calculations[3], and a distorted *cis* amide bond was predicted from these calculations. Whilst our work was in progress, it was reported that a heptapeptide (3) containing the same cyclic disulphide adopts two conformations in DMSO[4]. The major conformation has a *cis* amide bond in the Cys-Cys unit, and the minor conformation a *trans* amide bond. However, the enantiomer of disulphide (1) present in the naturally occuring bicyclic peptide malformin A has been proposed to exist in the *trans* form[5], and two related eight membered ring disulphides have also been found to contain *trans* amide bonds[6]. Thus it appears that a cyclo-[cysteinyl-cysteine] derivative can contain either a *cis* or *trans* amide bond depending upon the absolute configuration and substitution pattern of the eight membered ring.

Inspection of molecular models, revealed that for ring sizes less than eight, an amide in the ring must adopt the *cis* configuration whilst for rings with nine or more atoms, a *trans* amide can be accommodated and would be expected to be prefered. The change over occurs with eight membered rings, where the amide bond geometry will be determined by the other groups present in the molecule. Thus by studying eight membered ring disulphides, the relative importance of these factors can be determined. In this paper, our results on the conformation adopted by *N*-BOC-*cyclo*-[(**R**)-cysteinyl-(**R**)-cysteine] *t*-butyl ester (4) in solution are presented.

Compound (4) was prepared from *S*-trityl-(**R**)-cysteine by the literature procedure[7], and obtained as a white powder after recrystallization from methanol. This material eluted as a single peak under two sets of HPLC conditions.

NMR Results

The nmr spectrum of compound (4) was examined in three solvents, chloroform, dimethylsulphoxide, and acetone. The very low solubility in acetone prevented any useful information being obtained from that solvent, the results for the other two solvents are discussed below.

The 250MHz ^{1}H nmr spectrum of compound (4) in $CDCl_3$ was complicated due to overlap of the CH_2S protons, and to the α-CH peaks not being first order. However, evidence of at least two distinct conformations could be found as for some resonances (both ^{t}Bu, 1 α-CH, both NH's) two sets of peaks were present. The relative intensities of the two conformers at 17°C were 70/30. However, the peaks for the minor conformer were all very broad and showed no couplings. At 600MHz however the spectrum was almost entirely first order, and more evidence of a second conformation could be seen. Again all of the minor conformer peaks were broadened. The 62MHz ^{13}C nmr spectrum (in $CDCl_3$) by comparison showed only a single set of resonances, probably due to the minor conformer signals not being visible above the signal to noise ratio. As it was intended to use nmr techniques to carry out a conformational analysis of compound (4), an essential first step was to assign all of the peaks in the nmr spectra. However, the almost symmetrical nature of the compound meant that all of the peaks in the ^{1}H spectrum and all but one of the peaks in the ^{13}C spectrum could be assigned to either of two nuclei. The only exception was the ^{13}C resonance at 155ppm which could be unambiguously assigned to the urethane carbonyl. This assignment was of value in assigning the remaining peaks in the spectrum *via* 1 and 2/3 bond proton-carbon correlations.

The 600MHz 2/3 bond inverse detected proton carbon correlation (HMBC)[8] spectrum is shown in Figure 1, and the corresponding 1 bond correlation (HMQC)[9] spectrum in Figure 2. The correlations and resulting assignments for the major conformer are shown in Figure 3. From Figure 1 it can be seen that only one of the αH's (4.6ppm) shows a correlation to the urethane carbonyl (C10) resonance at 155ppm. Thus this must be H7 as shown in Figure 3. This proton also shows a correlation to the carbon resonance at 172ppm, allowing this to be assigned to the amide carbonyl (C6). The other α-proton (H4, 4.8ppm) does not show a correlation to the urethane carbonyl (C10) but does correlate to both the amide (C6) and ester (C11) carbonyl resonances at 172 and 168ppm respectively, as would be expected. Similarly, one of the NH resonances (at 5.9ppm) correlates to both the urethane carbonyl (C10) and the amide carbonyl (C6) allowing it to be assigned to the urethane NH (H9), whilst the other NH (at 6.25) correlates only to the amide carbonyl (C6) allowing it to be assigned as the amide NH (H5). Of the four CH_2S protons, the one resonating at 2.5ppm shows a correlation to the ester carbonyl (C11) allowing it to be assigned to one of the diastereotopic H3's.

Figure 1: Long Range Proton-Carbon Correlation of Compound (4) in $CDCl_3$.

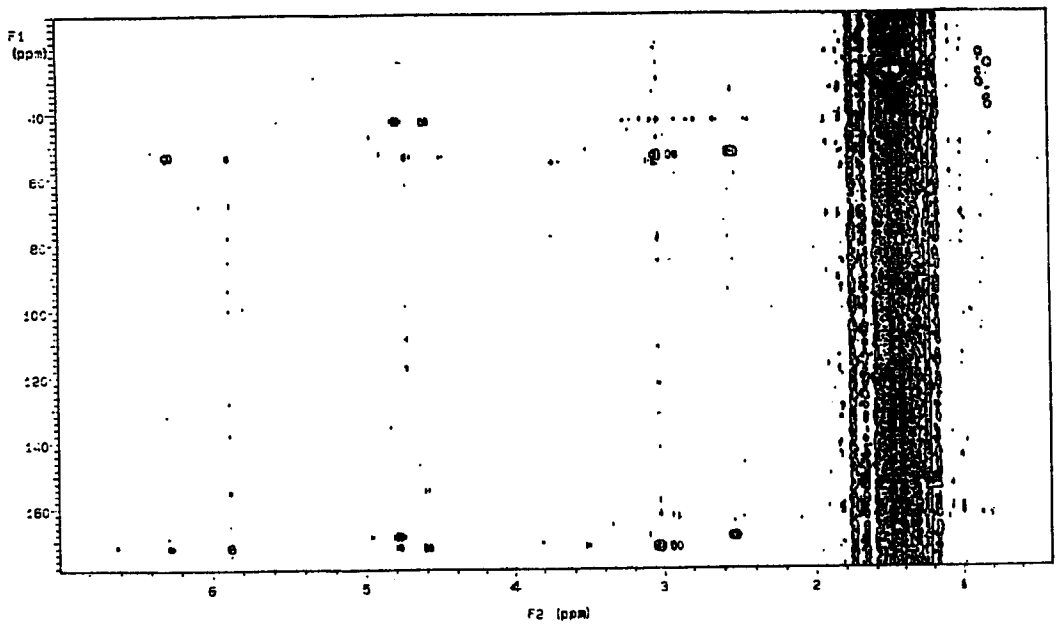
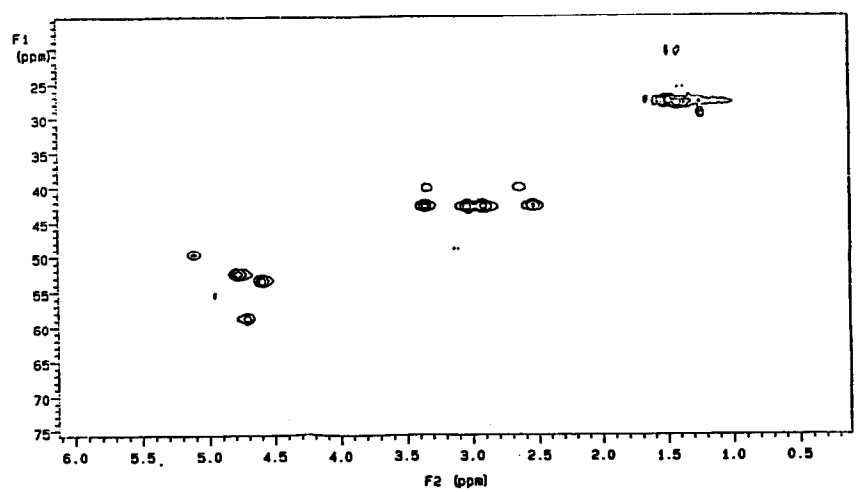
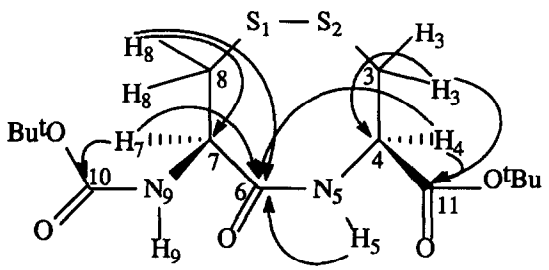


Figure 2: One Bond Proton-Carbon Correlation of Compound (4) in $CDCl_3$.



This proton also shows a correlation to the α-carbon at 50ppm, allowing it to be assigned to carbon (C4). The next two CH_2S resonances (at 2.9 and 3.05ppm) both correlate to the amide carbonyl (C6) but not to the ester carbonyl, allowing them to be assigned to the diastereotopic H8 protons. Both of these protons also correlate to the α-carbon at 52ppm allowing it to assigned to carbon (C7). The remaining CH_2S proton at 3.4ppm shows no long range proton carbon correlations, but by a proccess of elimination must be the other H3 proton. The two β-carbons (C3 and C8) have coincident resonances at 43ppm, thus all of the resonances corresponding to the major conformer in the proton and carbon spectra of compound (4) have been assigned except for the diastereotopic H3 and H8 protons.

Figure 3: Two and Three Bond Proton-Carbon Correlations.



The HMQC spectrum (Figure 2) then provides confirmatory evidence of these assignments since the proton at 4.6ppm (H7) correlates to the carbon at 52ppm (C7) and the proton at 4.8ppm (H4) correlates to the carbon at 50ppm (C4) as expected. In addition, the HMQC spectrum also shows correlations for the minor conformation not seen in the HMBC spectrum due to the signal to noise ratio. Evidence for two minor α-CH's is apparent from the protons at 4.7ppm (correlated to a carbon at 59ppm), and at 5.1ppm (correlated to a carbon at 50ppm). In addition, evidence for two minor β-protons is seen, both coupled to a carbon at 40ppm. These minor resonances are visible in the 600MHz ^1H spectrum, but not in the 62MHz carbon spectrum presumably due to an inadequate signal to noise ratio.

The above assignments were also consistent with a 250MHz ^1H-^1H correlation. The higher frequency NH (6.2ppm, assigned to the amide H5) was correlated to the higher frequency α-CH (4.8ppm, H4), which was in turn correlated to the highest (3.4ppm) and lowest (2.5ppm) frequency β-protons (H3). These two β-protons also correlate to one another (and not to the central β-protons). Correspondingly, the lower frequency NH (5.9ppm, urethane NH9) correlates to the lower frequency α-CH (4.6ppm, H7), which correlates to the central β-protons (3.0ppm, H8).

The remaining protons in the minor conformer were assigned by 600MHz TOCSY[10] spectra recorded with mixing times of 90ms and 120ms and shown in Figure 4. The minor amide resonance at 6.6ppm shows an exchange correlation to the major amide resonance at 6.2ppm, allowing it to be assigned to H5 of the minor conformer. Correlations are then seen to the H4 resonances of both the major (4.8ppm), and minor (4.7ppm) conformers, and to the H3 resonances of both conformers (3.4 and 2.5ppm for the major conformer, and 3.1 and 3.0ppm for the minor conformer). This coupling pattern is mirrored in the cross sections

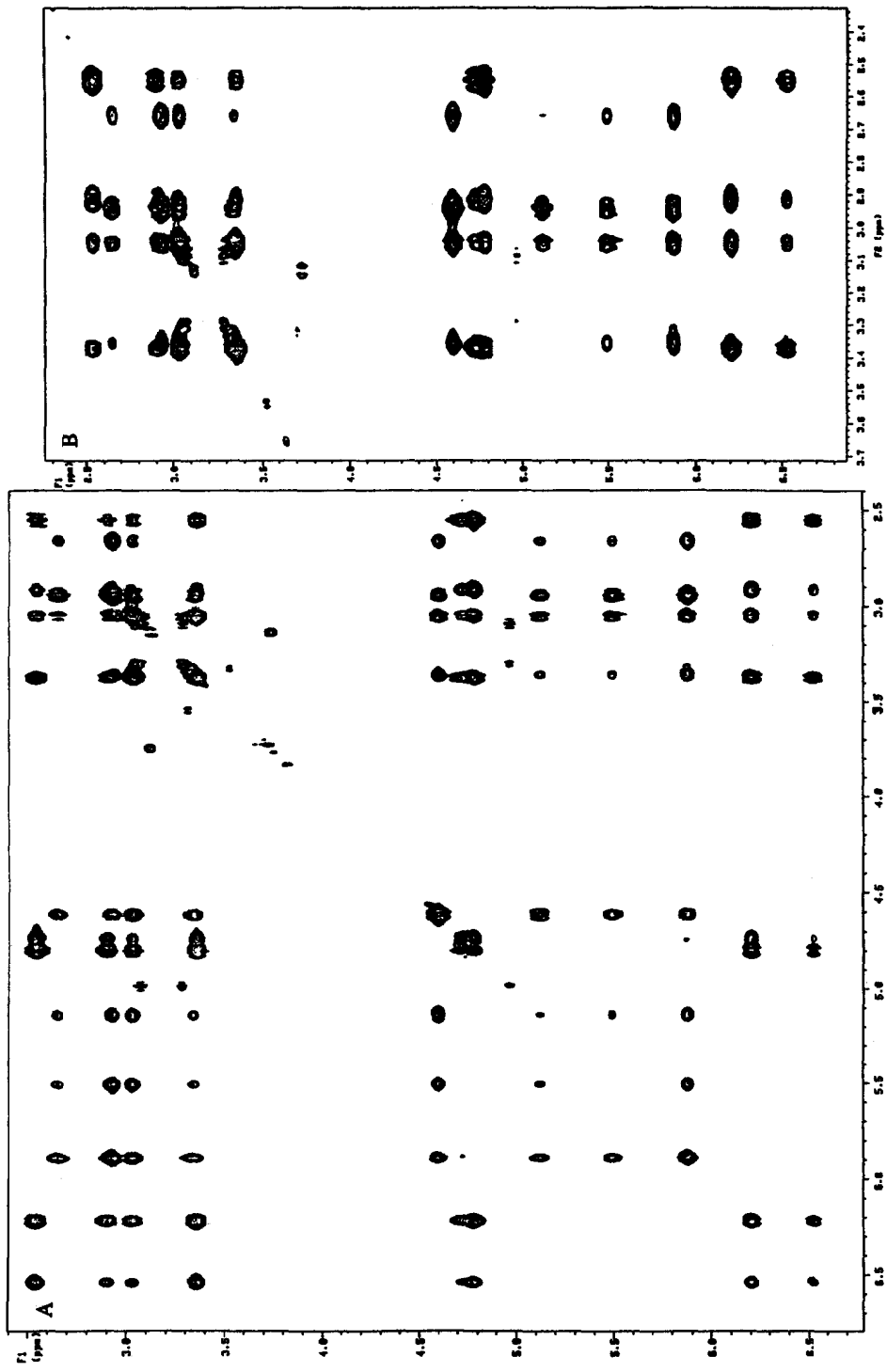


Figure 4: 600MHz TOCSY Spectra of Compound (4). A with a Mixing Time of 90ms; B with a Mixing Time of 120ms.

corresponding to each of the major and minor H3, H4 and H5 proton resonances. The minor amide resonance at 5.6ppm shows an exchange correlation to the major amide resonance at 5.9ppm corresponding to H9, allowing it to be assigned to H9 of the minor conformer. Correlations are then seen to the major (4.6ppm) and minor (5.1ppm) H7 resonances, and to the H8 resonances at 2.9 and 3.0ppm for the major conformer, and 3.4 and 2.7ppm for the minor conformer. Again this coupling pattern is mirrored in the cross sections corresponding to each of the H7, H8, and H9 protons. Thus every resonance in the ^1H nmr spectrum of compound (4) could be assigned, even in the region of the CH_2 protons where there is extensive signal overlap.

Having assigned the various proton and carbon resonances, conformational information could be obtained. The coupling constant between the amide NH5 and the α-CH4 is 11.5Hz for the major conformer, this value indicates a dihedral angle of approximately 165° between these two protons[11]. However the main factor that can influence the conformation of the ring is the amide bond geometry, and this cannot be determined directly from coupling constants. Inspection of a molecular model of compound (4), with the H4-H5 angle fixed at about 165°, indicated that a *cis* amide bond would bring the two α–protons (H4 and H7) into close proximity (200pm), whilst a *trans* amide bond would force them far apart (500pm). Thus it was anticipated that an n.O.e experiment would allow this geometry to be determined, provided that the two α-protons could be selectively irradiated[12].

n.O.e. Results

Attempts to record 600MHz n.O.e. spectra of compound (4) at room temperature were hampered by extensive magnetisation transfer between the two (and possibly a third) conformations. However this magnetisation transfer did allow further conformation of the peak assignments obtained from the HMBC, HMQC, and TOCSY spectra. By conducting the n.O.e. experiments at -20°C however, this magnetisation transfer was much reduced, and genuine n.O.e. information could be obtained. Irradiation of the resonances corresponding to NH5 and NH9 of both the major and minor conformer showed no significant enhancement in resonances elsewhere in the spectrum.

By contrast, irradiation of the H4 resonances of the major and minor conformer showed in both cases an enhancement of 5% in the resonances corresponding to the H7 protons of the major and minor conformer respectively. These results were mirrored when the H7 proton resonances were irradiated; the minor conformer showed a 5% enhancement in the minor conformer H4 resonance, and the major conformer a 4% enhancement in the major conformer H4 resonance. These results clearly indicate that both the major and minor conformers of compound (4) possess a *cis* amide bond. It should be noted that the n.O.e. spectra recorded at room temperature showed the same magnitude of n.O.e. between H4 and H7, but magnetisation transfer between the major and minor conformations was three times greater. Hence, as the size of the observed n.O.e. between H4 and H7 is independent of the amount of magnetisation transfer between the major and minor conformation, the observed n.O.e.'s must represent genuine enhancements for both conformations. Irradiations at the H4 and H7 resonances showed no n.O.e. enhancements at any of the H3 or H8 resonances.

Irradiation of the H3 and H8 resonances was complicated by the extensive signal overlap that occurs in this region of the spectrum. Only in the case of the major conformer H3 resonance at 2.5ppm, and the minor conformer H8 resonance at 2.6ppm was selective irradiation possible. In the later case, the only significant

enhancement was to the minor conformer geminal H8 resonance at 3.4ppm. Irradiation of the H3 resonance at 2.5ppm however showed a 5% enhancement in the geminal H3 resonance at 3.4ppm, and a 1% enhancement in the H4 resonance at 4.8ppm.

In summary, the n.O.e. results show that both conformers of compound (4) present in chloroform solution have a *cis* amide bond. However, the absence of n.O.e. enhancements between the α and β protons, the extensive signal overlap in the CH_2 region of the spectrum, and the magnetisation transfer between the two conformers prevented any further conformational information from being obtained.

Variable Temperature nmr Results

A variable temperature study of compound (4) in $CDCl_3$ was carried out at 250MHz over the temperature range of -55°C to +60°C, and at two concentrations; saturated (c.a. 30mg in 0.5ml), and dilute (5mg in 0.5ml), and a number of changes were noted in the spectra. At temperatures of 0°C or lower, the resolution of the spectra increases markedly, this is particularly apparent for the minor conformer which at room temperature gives only broad unresolved peaks. At lower temperatures however, the peaks become well resolved. Conversely, when the temperature is increased above room temperature, all of the peaks in the spectrum become less well resolved, until at 60°C only broad peaks are observed even for the major conformer. These results suggest that the two conformations observed for compound (4) are interconverting on the nmr time scale, which at room temperature results in some line broadening. When the temperature is reduced, the rate of interconversion decreases and the peaks become better resolved. However when the temperature is increased, the rate of interconversion increases, and all of the peaks become broadened. The high concentration variable temperature nmr data is summarised in Tables 1 and 2.

As can be seen from Tables 1 and 2, there are also chemical shift changes when the temperature is varied. For the major conformer, H5 the amide NH shifts downfield as the temperature is decreased (0.014ppm/K at high concentration). This is consistent with the formation of a hydrogen bond involving this proton at low temperature. As there are no significant changes in any of the coupling constants over this temperature range, it is likely that an intermolecular hydrogen bond is formed, hence at low temperature and high concentration in $CDCl_3$, the major conformer of compound (4) appears to form a hydrogen bonded network through the amide NH's. Additional evidence for this comes from the fact that when the concentration is reduced, H5 varies much less with temperature (6.14ppm at 20°C to 6.50ppm at -50°C; 0.005ppm/K). For the minor conformer by comparison, there is only a small change in the chemical shift of H5 as the temperature decreases (0.003ppm/K), and this is not significantly dependent upon the concentration. However as the resolution of this resonance increases at low temperature, the H4-H5 coupling constant of 8.1Hz can be obtained. This corresponds to a dihedral angle of 155° between these two protons[10].

The urethane NH (H9) of the major conformer shows only a small shift downfield as the temperature is decreased (0.002ppm/K), and this is independent of concentration. By contrast however, the corresponding resonance in the minor conformer shows a much larger downfield shift at high concentration (0.007ppm/K), but this is concentration dependent, being reduced to 0.003ppm/K at low concentration. This could be due to formation of a hydrogen bonded network involving the urethane NH in the minor conformer at low temperature.

Table 1: Variable Temperature NMR data for the Major Conformer of Compound (4) as a Saturated Solution in Chloroform.

Temp.°C	H5	H9	H4	H7	H3	H8	H8'	H3'
60	6.14ppm broad	5.69ppm broad	4.75ppm multiplet	4.61ppm multiplet	3.2ppm broad	3.2ppm broad	2.84ppm broad	2.68ppm broad
50	6.14ppm broad	5.78ppm broad	4.75ppm multiplet	4.59ppm multiplet	3.2ppm broad	3.2ppm broad	2.84ppm broad	2.64ppm broad
40	6.14ppm broad	5.85ppm broad	4.78ppm multiplet	4.59ppm multiplet	3.35ppm broad	3.07ppm broad	2.91ppm broad	2.59ppm broad
17	6.14ppm d 11.5Hz	5.88ppm d 5.7Hz	4.78ppm t 11.5Hz	4.59ppm broad	3.38ppm d 13.1Hz	3.05ppm d 15.0Hz	2.92ppm multiplet	2.52ppm t 12.6Hz
0	6.49ppm d 11.7Hz	5.89ppm d 7.0Hz	4.78ppm dt 12.0, 4.0Hz	4.59ppm multiplet	3.31ppm dd 13.3, 3.8Hz	2.99ppm multiplet	2.99ppm multiplet	2.50ppm t 12.6Hz
-10	6.58ppm d 12.0Hz	5.89ppm d 7.0Hz	4.78ppm dt 11.8, 3.7Hz	4.59ppm multiplet	3.32ppm dd 13.4, 3.8Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.4Hz
-20	6.70ppm d 11.9Hz	5.90ppm d 7.0Hz	4.78ppm dt 11.8, 3.7Hz	4.59ppm multiplet	3.35ppm dd dd 13.4, 3.6Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.6Hz
-30	6.83ppm d 11.5Hz	5.94ppm d 7.0Hz	4.78ppm dt 11.8, 3.7Hz	4.59ppm multiplet	3.34ppm dd dd 13.4, 3.6Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.6Hz
-40	6.96ppm d 11.9Hz	5.96ppm d 6.9Hz	4.78ppm dt 11.9, 3.5Hz	4.59ppm multiplet	3.35ppm dd dd 13.2, 3.5Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.6Hz
-50	7.10ppm d 11.8Hz	5.96ppm d 6.9Hz	4.78ppm dt 11.9, 3.8Hz	4.59ppm multiplet	3.32ppm dd dd 13.2, 3.4Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.6Hz
-55	7.18ppm d 11.8Hz	5.96ppm d 7.0Hz	4.78ppm t 11.9Hz	4.59ppm multiplet	3.32ppm dd d 13.1, 3.2Hz	2.94ppm multiplet	2.94ppm multiplet	2.50ppm t 12.6Hz

Quoted coupling constants are as they appear in the spectrum, and have not been corrected for second order effects. The peaks are assigned as H1-H9 as shown in Figure 3.

The α-protons (H4 and H7) show no significant temperature dependence of their chemical shift for either conformer at either concentration. The β-protons (H3 and H8) show small changes in chemical shift as the temperature is varied as shown in Table 1 and Table 2.

Table 2: Variable Temperature NMR Data for the Minor Conformer of Compound (4) at High Concentration in Chloroform

Temp.ºC	H5	H9	H7	H8	H8'
30		5.43ppm broad			
17	6.50ppm broad	5.48ppm broad	5.10ppm broad		2.63ppm broad
0	6.59ppm d 8.1Hz	5.49ppm d 9.3Hz	5.09ppm multiplet	3.31ppm hidden	2.63ppm t 11.8Hz
-10	6.61ppm hidden	5.64ppm d 9.3Hz	5.08ppm dt 5.6,4.0Hz	3.31ppm hidden	2.63ppm t 11.9Hz
-20	6.62ppm hidden	5.70ppm d 9.3Hz	5.08ppm dt 5.5,4.0Hz	3.30ppm hidden	2.65ppm t 12.0Hz
-30	6.68ppm d 8.1Hz	5.79ppm d 9.3Hz	5.08ppm dt 5.6,4.0Hz	3.29ppm hidden	2.69ppm t 11.9Hz
-40	6.73ppm d 8.1Hz	5.88ppm d 9.3Hz	5.08ppm dt 5.6,4.0Hz	3.28ppm hidden	2.69ppm t 11.9Hz
-50	6.73ppm d 8.2Hz	5.95ppm d 9.3Hz	5.08ppm broad	3.27ppm hidden	2.70ppm t 11.8Hz
-55	6.75ppm d 8.1Hz	5.95ppm d 9.3Hz	5.08ppm broad	3.22ppm hidden	2.70ppm t 11.6Hz

The peaks are assigned as H1-H9 as shown in Figure-3. The other minor conformer resonances were hidden beneath major conformer peaks over the whole temperature range. Quoted coupling constants are as they appear in the spectrum, and have not been corrected for second order effects. A blank indicates that the resonance could not be observed at that temperature, this is true of all minor conformer peaks at temperatures above 30°C. Hidden indicates that the peak was partially obscured by a major conformer peak, so its coupling constants could not be determined.

NMR Results in DMSO

The ^1H nmr spectrum of compound (4) in DMSO was far more complex than the corresponding spectrum in $CDCl_3$. Examination of the NH region (6-9ppm) of the spectrum of a saturated solution of compound (4) in DMSO gave evidence of at least three conformations, which are of approximately equal intensity, in contrast to the situation in $CDCl_3$. However, this caused the α-H and β-H regions of the spectrum to be extremely complex even at 400MHz due to extensive signal overlap between the three conformations. The ^1H nmr spectrum in DMSO was found to be concentration dependent, at low concentration only NH peaks corresponding to two conformations were observed, whilst at high concentrations six NH resonances were observed, corresponding to at least three conformations. Furthermore this is not due to peaks for the third conformer simply not being seen at low concentration due to the signal to noise ratio, as the peaks of highest intensity in the high concentration spectrum were absent from the low concentration spectrum.

The ^{13}C nmr spectrum also indicated the presence of three conformations as multiple signals were observed for each resonance as shown in Table 3. Hence the spectra in DMSO indicate the presence of three conformations of compound (4), whereas in $CDCl_3$, only two conformations are detected.

Table 3: ^{13}C NMR Chemical Shifts of Compound (4) in DMSO.

Carbon No.	Chemical Shifts (ppm)
C6	172.20, 171.58, 170.89
C11	169.10, 168.87
C10	155.46, 155.13, 154.78
2*$OCMe_3$	81.68, 80.77, 79.15, 78.75, 78.62, 78.51
C4 and C7	53.93, 53.69, 52.48, 51.31, 49.35
C3 and C8	Hidden beneath the DMSO peak
2*$(CH_3)_3$	28.32, 28.27, 28.23, 27.77, 27.74, 27.71

Spectrum recorded at 100MHz.

Solid State NMR Results

In an attempt to correlate the solution conformations of compound (4) with the conformation determined by X-ray crystallography, a solid state ^{13}C nmr spectrum was obtained. It was hoped that any differences between the solution and solid state conformations would show up as changes in ^{13}C chemical shifts between the two states. As is shown in Table 4, only small differences between the two sets of chemical shifts were observed, this could be due either to the two conformations being comparable, or to ^{13}C chemical shifts being insufficiently sensitive to changes in conformation. Hence this experiment was largely inconclusive.

Table 4: Solution and Solid State ^{13}C NMR Chemical Shifts of Compound (4) at 62.5MHz.

Assignment	Solution Chemical Shift (ppm)	Solid State Chemical Shift (ppm)
C6	172.2	173.7
C11	168.9	170.1
C10	154.7	154.3
$OCMe_3$	83.8 and 80.2	82.8 and 81.1
C7	53.5	52.1
C4	52.6	50.6
C3 and C8	42.8	43.5 and 41.6
$(CH_3)_3$	28.3 and 27.9	29.1

Molecular Modelling Results

In an attempt to obtain further information on the conformations available to compound (4), a molecular mechanics study of this compound was carried out using the Macromodel 3D program[13]. A randomly drawn conformation of compound (4) was energy minimised, and then used as a starting geometry for a grid search of all possible conformations conducted at 60° resolution. During this search, all of the bonds within the eight membered ring were rotated, as were the C_α-CO bond of the ester, and the C_α-NH-CO bonds of the urethane. Conformers having unreasonable bond lengths within the eight membered ring (S-S<100pm, or S-S>250pm) were automatically rejected by the program, and the remainder were energy minimised to give a set of minimum energy conformations. All conformations within 50kJmol^{-1} of the global minimum were kept for further study. The energy minimisation was carried out *in vacuo*, using three different forcefields MM2,

AMBER, and OPLSA. In addition, energy minimisation using the MM2 and AMBER forcefields was carried out in simulated chloroform and water solvents as generated by the Macromodel program.

However, in every minimisation run, the program predicted that conformers with a *trans* amide bond in the eight membered ring would be 12-16 $kJmol^{-1}$ more stable than conformers with a *cis* amide bond, a prediction that is clearly not compatible with the nmr results. One possible explanation for this was that a grid search at 60° resolution is inadequate for compound (4) where because of the presence of an eight membered ring, conformations differing in torsional angles by less than 60° might be envisaged. In order to overcome this, Monte Carlo searches were carried out on compound (4), using the lowest energy *cis* and *trans* conformers as the starting geometries. The searches were allowed to run for 5,000 iterations and used the 'least usage search directed' algorithm within the Macromodel program to ensure uniform coverage of the entire conformational space. This search was carried out with the MM2 and AMBER forcefields, either *in vacuo*, or using a simulated chloroform solvent. As expected, far more conformations were found in this way, however conformations with a *trans* amide bond were again about $15kJmol^{-1}$ more stable than those with a *cis* amide bond.

A second possible explanation for the prediction of a *trans* amide bond when a *cis* amide is observed, was that the calculations were carried out on just one molecule of compound (4), however, the variable temperature nmr results indicate that at least at low temperature an intermolecular hydrogen bonded structure is formed. In order to model this, a Monte Carlo search was initiated in which two identical molecules of compound (4) with *cis* amide bonds were used as starting geometries. The two molecules were initially located close to one another so that intermolecular hydrogen bonds could form between the amides. The Monte Carlo search was then initiated as described above, with the two molecules free to move relative to one another, however the minimum energy structure was found to consist of two identical molecules each with a *trans* amide bond and hydrogen bonded together through both the amide and urethane carbonyls and NH's.

Examination of the various *cis* conformers that were found during the single molecule minimisations, revealed that the first fifteen of these covered a $30kJmol^{-1}$ energy range, and all had one of three ring conformations A-C as shown in Table 5. Within each family, the conformers differ in the orientation of the ester and urethane groups. Of the three families, B had a ring conformation in which the two α-protons were not close to one another, instead the ring was twisted so that H7 was close to one of the H3 protons. This is clearly not compatible with the n.O.e. experiments, and the predicted coupling constants for H4-H5, and H3-H4 for this conformer are also not compatible with those observed, so this conformation will not be discussed further. Conformers of type A and C are shown in Figure 5, they differ mainly in the helicity of the disulphide bond. Conformer C was found to have an identical ring conformation to that observed for compounds (1) and (2) by X-ray crystallography[2]. The predicted coupling constants for H3-H4 for both conformer type A and C agree well with those observed for the major conformer in solution (Table 1). The dihedral angle between H4 and H5 for conformer A is calculated as 140°, and for C as 159°, which are compatible with the values of 165° and 155°, observed for the major and minor conformers respectively in chloroform. Hence it is possible to deduce that the major conformer present in chloroform solution corresponds to either structure A or C. The other conformer observed in chloroform would then be compatible with the other structure A or C. However, far less nmr data is available for the minor conformer, due to the peaks being hidden under the major conformer resonances, and being broad at higher temperatures. It is not possible to state for certain which of the conformations A or C corresponds to the major conformer in chloroform solution, but the observed

dihedral angle for H4-H5, and the magnitude of the smaller H3-H4 coupling constant are more consistent with structure C, the X-ray structure than with conformers of type A.

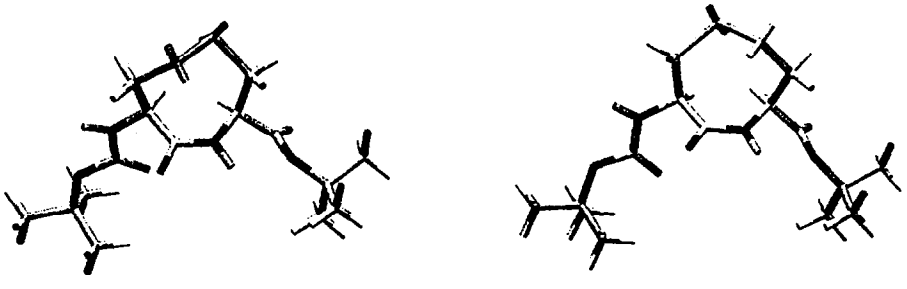
Table 5: Predicted Conformers of Compound (4) with a Cis Amide Bond.

Conformer No.	Relative Energy (kJmol^{-1})	Ring Family	Predicted J (H3-H4)[a]
1	0	A	11.4, 1.7
2	1.39	A	11.4, 1.7
3	6.22	A	11.4, 1.7
4	11.18	A	11.3, 1.6
5	12.71	A	11.4, 1.7
6	13.52	A	11.3, 1.6
7	15.07	A	11.4, 1.7
8	15.78	A	11.4, 1.7
9	18.20	A	11.4, 1.7
10	18.54	B	4.4, 2.6
11	20.30	B	4.8, 2.4
12	26.12	C	11.7, 3.9
13	26.42	B	4.5, 2.6
14	28.18	C	11.7, 4.0
15	28.20	B	4.9, 2.3

a) Coupling constants computed within the Macromodel program. The calculations were carried out in a simulated chloroform solvent using the Amber forcefield.

Figure 5: Conformer Type A and C Predicted for Compound (4) by Molecular Mechanics.

1) 2)



1): Conformer A calculated to be of lowest energy. 2): Conformer C corresponding to the X-ray structure.

Conclusions

Compound (4) was found to exist in chloroform as a mixture of two conformers, and in DMSO solution as a mixture of three conformers interconverting slowly on the nmr time scale. In chloroform, the two conformers have a *cis* amide bond and differ mainly in the helicity of the disulphide. The major conformer present in chloroform solution seems to have the same ring geometry as found in the crystal structure of similar compounds[2]. Evidence was found that in chloroform, the compound forms intermolecular hydrogen bonds at least at low temperature. Molecular mechanics calculations were found to be unable to predict the relative energies of the minimum energy conformations of compound (4), but did find the experimentally observed conformations (including the conformation found by X-ray crystallography) amongst the set of conformations predicted.

Experimental

NMR experiments were carried out at 250MHz on a Brucker AM250 spectrometer fitted with a 5mm ^{1}H-^{13}C dual probe or a solid state probe; at 400MHz on a Brucker WH400 spectrometer; or at 600MHz using a Varian VXR600S spectrometer.

Molecular modelling calculations were carried out using Macromodel-3D running on a Silicon Graphics Personal Iris Workstation.

Acknowledgements

The authors would like to thank the SERC for use of the 400MHz nmr service at the University of Warwick (and especially thank Dr. O. Howarth for his helpful advice), the 600MHz nmr service at the University of Edinburgh, and the mass spectroscopy service at the University of Wales; University College of Swansea. The authors also thank the Wolfson Foundation for a grant to the IMBE at Bangor, from which the Silicon Graphics computer was purchased. AH thanks the EEC social fund for a studentship, and MN thanks the University of Wales; University College of North Wales for a research grant.

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12) In the case of the heptapeptide described in reference 4, the geometry of the amide bond was determined *via* a NOSEY experiment which avoided the need to selectively irradiate two close protons. However when this was attempted for compound (4), only a low quality spectrum was obtained even at 600MHz, presumably due to the molecule being too small and hence tumbling too fast to show significant NOSEY cross peaks. In addition, the presence of a large number of cross peaks due to conformational exchange further complicated the spectrum.

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