Cyclic Pentapeptides of Sarcosine in Combination with Either Alanine or Glycine. Syntheses and Conformational Processes Studied by NMR Spectroscopy

KIRSTEN TITLESTAD

Kjemisk Institutt, Universitetet i Oslo, Blindern, Oslo 3, Norway

A series of cyclic pentapeptides has been synthesised. Cyclic dipeptides, tetrapeptides and partially racemised tetra- and pentapeptides were isolated as by-products. The formation of the smaller rings is due to cleavage of the peptide chain during the cyclisation reaction.

NMR studies of the cyclic pentapeptides in CHFCl₃ showed that several conformational changes took place when solutions prepared by dissolving crystals at −70 °C were gradually heated to +20 °C. The conformers present in the final equilibrium, usually reached below room temperature, varied for the different cyclic pentapeptides and the initial crystal conformer was hardly present.

The driving force behind these conformational changes is suggested to be replacement of external hydrogen bonds in the crystal conformer with internal hydrogen bonds to form more stable conformers in solution. This requires conversion of cis amide bonds to trans. Attempts were made to distinguish between cis and trans amide bonds by the following NMR methods: differential solvent shifts, different band widths of the N-methyl lines, shifts induced by complexation with Eu, and shift differences in ¹³C NMR, but no conclusive results could be obtained. Addition of benzene, was, however, helpful in resolving overlapping lines.

The majority of conformational studies on cyclic peptides have been carried out on cyclic dipeptides and cyclic hexapeptides, only a few isolated investigations have been reported for cyclic pentapeptides. A difference was found in the yield on cyclisation of glycylleucyclidiglycylleucine and of glycylidiglycylleucine and also higher yields when both D- and L-leucine was used when compared to the L,L-isomer. Dielectric increment measurements showed a shorter distance between the ends of the linear peptides containing the L,D-form and this would favour the cyclisation. Five cyclic pentapeptides such as cyclo-glycyl-L-alanyldiglycyl-L-prolyl and similar compounds were studied by NMR spectroscopy in dimethyl sulfoxide solution and found to be present in two conformations, about 70 % of the major and 30 % of the minor conformer. It was suggested that the major conformer contained only trans amide bonds whereas the minor conformer had the amide bond involving the proline nitrogen in the cis form. Both conformations contained intramolecular hydrogen bonds.

Our work with cyclic homologues of sarcosine has shown that these cyclic peptides have a high degree of conformational homogeneity and most of the rings are present in solution in only one conformation. It was also found that a series of cyclic tetrapeptides of sarcosine, in combination with one or two alanine or glycine residues, take the same conformation as the parent compound, cyclo-tetrasarcosyl. In these, the configuration of the four amide groups are alternately cis and trans. This skeleton was adopted even in those cases where an NH-amide had to take a cis configuration. The corresponding cyclic penta-peptide, cyclo-pentasarcosyl, when studied by NMR spectroscopy in deuteriochloroform, revealed one major and one minor (less than 10 %) conformation. Low-temperature NMR studies showed the major conformation to be the same as that in the crystal with the con-
Scheme 1. Routes to the different linear pentapeptides.

For cyclo-L-alanyltetrasarcosyl

<table>
<thead>
<tr>
<th>L-Ala</th>
<th>Sar</th>
<th>Sar</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td></td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
</tr>
</tbody>
</table>

For cyclo-D-alanyl-L-alanyltrisarcosyl

<table>
<thead>
<tr>
<th>Sar</th>
<th>D-Ala</th>
<th>L-Ala</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
<td>Z</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td>Z</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
</tr>
</tbody>
</table>

For cyclo-di-L-alanyltrisarcosyl

<table>
<thead>
<tr>
<th>Sar</th>
<th>Sar</th>
<th>L-Ala</th>
<th>L-Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>Z</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td>OMe</td>
</tr>
</tbody>
</table>

For cyclo-L-alanylsercosyl-D-alanyldisarcosyl

<table>
<thead>
<tr>
<th>Sar</th>
<th>Sar</th>
<th>L-Ala</th>
<th>Sar</th>
<th>D-Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>Z</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td></td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td></td>
<td>OMe</td>
</tr>
</tbody>
</table>

For cyclo-L-alanyl-D-alanyl-L-alanyldisarcosyl

<table>
<thead>
<tr>
<th>L-Ala</th>
<th>D-Ala</th>
<th>L-Ala</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td></td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
</tr>
</tbody>
</table>

For cyclo-D-alanyl-L-alanylsercosyl-D-alanylsercosyl

<table>
<thead>
<tr>
<th>Sar</th>
<th>D-Ala</th>
<th>L-Ala</th>
<th>Sar</th>
<th>D-Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>OMe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For cyclo-glycyltetrasarcosyl

<table>
<thead>
<tr>
<th></th>
<th>Gly</th>
<th>Sar</th>
<th>Sar</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td></td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For cyclo-diglyclytrisarcosyl

<table>
<thead>
<tr>
<th></th>
<th>Sar</th>
<th>Gly</th>
<th>Gly</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>Z</td>
<td>OH</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td>Z</td>
<td>H</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For cyclo-glycylsarcosylglycyldisarcosyl

<table>
<thead>
<tr>
<th></th>
<th>Gly</th>
<th>Sar</th>
<th>Gly</th>
<th>Sar</th>
<th>Sar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OMe</td>
</tr>
</tbody>
</table>

figurational sequence cis,cis,cis,trans,trans,\(^5\,18\,4\) It was therefore considered to be of interest to study a series of cyclic pentapeptides of sarcosine combined with alanine or glycine to see whether these possessed the same degree of conformational homogeneity as found for the analogous tetrapeptides. The compounds prepared and studied were six cyclic pentapeptides of sarcosine combined with alanine:
cyclo-L-alanlytetrasarcosyl (c-L-AlaSar\(_4\)), cyclo-D-alanly-L-alanlytrisarcosyl (c-D-Ala-L-AlaSar\(_3\)),
cyclo-di-L-alanlytrisarcosyl (c-L-AlaSar\(_3\)), cyclo-L-alanlysarcosyl-D-alanlydisarcosyl (c-L-AlaSar-D-AlaSar),
cyclo-L-alanly-D-alanly-L-alanlydisarcosyl (c-L-Ala-D-Ala-L-AlaSar), cyclo-D-alanly-L-alanlysarcosyl-D-alanlysarcosyl (c-D-Ala-L-AlaSar-D-AlaSar),
and three cyclic pentapeptides of sarcosine in combination with glycine:
cyclo-glycyltetrasarcosyl (c-GlySar\(_4\)), cyclo-diglyclytrisarcosyl (c-GlySar\(_3\)), cyclo-glycylsarcosylglyclydisarcosyl (c-GlySarGlySar\(_2\)).

SYNTHESSES

The different linear peptides were synthesised as shown in Scheme I. The benzylxycarbonyl group was used for protection of the amino function, the methyl ester (in one case the t-butyl ester) for the carboxyl group and N,N'-dicyclohexylcarbodiimide as the coupling reagent. For cyclisation the methyl ester was hydrolysed by alkali (the t-butyl ester by trifluoroacetic acid) and the carboxy group converted to the 2,4,5-trichlorophenyl ester, the benzylxycarbonyl group was removed by hydrogenolysis and the peptide active ester cyclised in pyridine. After passage through a strong cat- and an-ion exchange column to remove noncyclised material, the cyclic peptides were purified by chromatography on silica gel, fractional crystallisation and sublimation. Not only the desired cyclic pentapeptide was isolated, but also dipeptides, tetrapeptides, decapeptides and racemised products (Table 1).

Formation of cyclic dipeptides was observed during cyclisation of peptides, especially those containing sarcosine as the second residue in the peptide chain. This was very prominent in a series of tetrapeptides \(^1\) and was also observed in the series of homologous sarcosine peptides.\(^8\) The free amino group attacks the second carboxyl carbon, with cleavage of the chain. Cyclic-
sation of alanylthriarcsosylsarcosine trichlorophenyl ester gave together with the expected product two cyclic dipeptides, cyclo-alanylsarcosyl and cyclo-disarcosyl (Table 1). Here the cleavage of the peptide chain has occurred twice as was suggested to be the case in some of the sarcosine peptides. However, from cyclisation of the closely related glycyllthriarcsosylsarcosine only the corresponding cyclic pentapeptide was isolated. This shows that cleavage of the peptide chain occurs, but less generally in pentapeptides than in tetrapeptides.11

The formation of cyclic tetrapeptides was observed only in the cyclisation of disarcosyl-L-alanlylsarcosyl-D-alanine trichlorophenyl ester where two cyclic tetrapeptides of sequence Ala Sar Ala Sar were isolated. These differed in solubility, sublimation temperature, IR and NMR spectra, and were assigned as cyclo-L-alanlylsarcosyl-D-alanlylsarcosyl (major product) and cyclo-L-alanlylsarcosyl-L-alanlylsarcosyl by comparison with the cyclic tetrapeptides previously studied.8 The possibility that these arise by transannular reactions from the two cyclic pentapeptides formed (see below for racemisation) was excluded as these were recovered unchanged after boiling in pyridine for 9 h. Most probably these tetrapeptides were again formed by cleavage of the peptide chain, but as in both cases it is the N-terminal sarcosine which is lost, the attack must be from the carbonyl end. At first sight it is difficult to rationalise the preference of the carbonyl carbon of the second residue over the free amino group. However, the relatively low yield of cyclic pentapeptide and relatively high yield of cyclic tetrapeptide would suggest that the linear molecule exists in a conformation which favours the formation of the smaller ring. Cyclo-L-alanlylsarcosyl-D-alanlylsarcosyl is known from a previous study to take up the highly favoured cis,trans,cis,trans configuration with the Cz methyl groups in favoured orientations.8 This fits very well for folding the chain so that the amide nitrogen comes close to the active ester, and formation of a cyclic intermediate as shown in Fig. 1 is possible. The cleavage of the active ester may occur in several ways, either by the departure of a phenolate ion or elimination of phenol if the α-H of the D-alanine residue is removed. Subsequent loss of the N-terminal sarcosine unit leads to the two cyclic tetrapeptides which are isolated on cyclising this specific pentapeptide.

Cyclic decapetide formation can be very predominant from pentapeptides as shown in a systematic study on analogues of gramicidin S.12-14 Earlier studies with homologous sarcosine peptides have shown that this doubling reaction is not very dominant even on cyclisation of disarcosylsarcosine, and a similar situation has now been observed for the pentapeptide series. In most cases the presence of the cyclic decapetide could only be inferred from the TLC. Attempts to isolate these small quantities did not succeed as they showed no tendency for crystallisation and were bound strongly to silica gel. Only for alanylthriarcsosylsarcosine was the cyclic decapetide isolated by precipitation.

 Racemisation was shown to have occurred by the isolation of pairs of pentapeptides (and tetrapeptides) from some of the reactions. Thus disarcosyl-L-alanlylsarcosyl-D-alanine trichlorophenyl ester gave two cyclic pentapeptides (identical m/e 355) which had different melting points and different RF values on TLC and could be separated on silica gel. A similar situation was found on cyclising sarcosyl-D-alanyl-L-alanlylsarcosyl-D-alanine trichlorophenyl ester where two cyclic pentapeptides were isolated with different melting points and RF values. As the two linear pentapeptides both contain D-alanine as the C-terminal amino acid it is likely that on cyclisation in the basic

Fig. 1.
medium at the elevated temperature (115 °C) some racemisation of the C-terminal amino acid takes place. The two cyclic pentapeptides formed in the former case, are therefore most likely cyclo-L-alanysarcosyl-D-alanyldisarcosyl, m.p. 274 °C (major product) and cyclo-L-alanysarcosyl-L-alanysarcosyl, m.p. 224 °C. This is in good agreement with the two cyclic tetrapeptides which also were formed in this cyclisation. The products in the second case have most likely an analogous relationship: cyclo-D-alanyl-L-alanyl-L-alanyl-L-alanyl-L-sarcosyl, m.p. (subl.) 317 °C (major product), and cyclo-D-alanyl-L-alanyl-L-sarcosyl-L-L-sarcosyl, m.p. 260 °C. From each of the other cyclisation reactions only one cyclic pentapeptide was isolated, even though the X-ray analysis of the expected cyclo-L-alanyl-ditetrasarcosyl showed that the crystal contained the racemate. It is likely that the racemate crystallised more easily than each of the optical antipodes and that the crude cyclised product is not fully racemised. The optical rotation is low, but this can depend on the conformations of the cyclic peptides. The same was noted on crystallising the analogous cyclic tetrapeptide cyclo-L-alanyl-L-alanyl-L-sarcosyl where the racemate was found in the crystal structure. Thus with both the pentapeptide and the tetrapeptide partial racemisation has taken place at some stage during the reaction sequence. Recently, it has been reported that racemisation does occur in reactions which had been thought to be racemisation free.

Physical and Spectral Properties of the Cyclic Pentapeptides

In contrast to the cyclic oligomers of sarcosine which have sharp melting points, several of the cyclic pentapeptides, after being isolated by column chromatography, melted over a wide range. However, the melting points became sharper after recrystallisation from a suitable solvent (e.g. acetone) by slow evaporation in contact with the air. The infrared spectra, showing broad absorptions, gave little information. The mass spectra showed the molecular ions as intense peaks and very similar fragmentation patterns. The NMR spectra were generally more complex than in the sarcosine series but again gave the most informative picture of the conformational situation. When dissolved in CDCl₃ at room temperature, the number of lines showed the presence of two or more conformations in equilibrium. The conformational interconversion barriers are high, as temperatures of around 100 °C are needed to obtain coalescence to a single set of lines. This means that at low temperatures (about -70 °C) the conformers, at least as defined by a given cis,trans-sequence, will have life-times of hours. It should therefore be feasible to observe the conformation present in the crystal when crystals are dissolved at low temperature (-70 °C) and the NMR spectrum recorded at this temperature.* Slow warming of the sample in the NMR tube to room temperature and recording of the NMR spectra will reveal if any conformational changes take place.

When the initially isolated cyclic pentapeptides of broad melting range were dissolved at -70 °C, the NMR spectra were complex and showed that several conformations were present, while the sharply melting crystals when dissolved at -70°C showed only one conformer (called A) at this temperature (Figs. 2-5). On allowing the latter samples to warm slowly to about -30 °C, new lines due to a second conformer (called B) began to appear. On further warming a third conformer (C) developed whilst the concentration of the crystal conformation A diminished. The full thermodynamic equilibrium, normally reached already below room temperature, consisted of a mixture of B and C and in some cases a fourth conformer D. Only in a few cases was the crystal conformer present at room temperature. The changes are most easily seen in the N-methyl region, the methylene protons, however, do not often give well resolved lines, but in those cases where the quartets are recognized the coupling constants are grouped around 14 and 18 Hz indicating that the Cα₄-torsional angles take up the anti, gauche pattern as seen for the oligopeptides of sarcosine.* The NH protons could not be ob-

---

* Rotation about the single bonds is not expected to be slowed down sufficiently at these temperatures, but in a series of related cyclic peptides, cyclic oligomers of sarcosine, both the Cα₄- and the NCα₄-torsion angles take up only a limited number of values. The Cα₄-torsion angles are either 180 or 60° and the NCα₄-torsion angles are grouped around 90°.
Fig. 2. The 100 MHz NMR spectrum of α-GlySar$_4$. Crystals were dissolved in CHFCl$_3$ at $-50^\circ$C (lower spectrum) and the solution allowed to warm up in the probe (NH-region left out). CDCl$_3$ was used as the solvent at the highest temperature ($20^\circ$C).
Fig. 3. The 100 NMR spectrum of d-Ala-L-Ala-Sar₂. Crystals were dissolved in CHFCl₃ plus traces of TFA at −60 °C (lower spectrum) and the solution allowed to warm up in the probe (NH-region left out), CDCl₃ was used as the solvent at the highest temperature (30 °C).

served due to the chemical shifts of the CHFCl₃ proton, the solvent used in these NMR studies, either pure or together with a small amount of trifluoracetic acid to increase the solubility. When studied in deuteriochloroform at room temperature the NH protons are close to the chloroform peak and the spin-spin coupling to the methine protons, which are hardly resolved, varies from 1 to 10 Hz.

The conformational changes of the individual cyclic pentapeptides are closely related, but the concentration of the transient conformers differ and also the conformers present at equilibrium. c-GlySar₄ (Fig. 2) shows when dissolved at -50 °C four N-CH₃ lines due to the presence of one conformer, A. The CH₃-quartets are partly resolved. At about -35 °C a second conformer B appears, its concentration is always less than that of A and decreases when a third conformer C appears. At room temperature the conformer mixture is dominated by C (80 %) with a small amount of A but hardly any of B. This equilibrium mixture was warmed further to observe coalescence phenomena. The CH₃-quartets of conformer C started to broaden at 30 °C, at about the same temperature (40 °C) as was found for c-Sar₄. Thus conformer C possesses the same high barrier to ring inversion as found in c-Sar₄.

c-AlaSar₄ (Fig. in Ref. 32) shows at -60 °C four N-CH₃ lines, but the C-CH₃ is split into two doublets. This indicates the presence of two conformers having N-CH₃ groups of iden-

Cyclic Pentapeptides

Fig. 5. The 100 MHz NMR spectrum of c-D-Ala-L-AlaSar-D-AlaSar. Crystals were dissolved in CHFCl₃ plus traces of TFA at −45 °C (lower spectrum) and the solution allowed to warm up in the probe (NH-region left out). CDCl₃ was used as the solvent at the highest temperature (20 °C).

tical chemical shifts. One of the conformers may represent the crystal conformation whereas the other may occupy the same ring skeleton, but with the C-CH₃-group inverted. The new set of four N-CH₃ lines due to conformer B becomes in this molecule equal to A in concentration before conformer C appears, and at room temperature C dominates (60 %), together with B (40 %), but only traces of A are present.

c-D-Ala-L-AlaSar₃ (Fig. 3) and c-Gly₃Sar₃ exhibit identical conformational changes, conformer B approaching A in concentration before C increases to become the dominating conformer. At about 0 °C a fourth conformer D develops and at room temperature the mixture consists of mostly D (70 %) and some C (30 %). The closely related c-L-Ala₃Sar₃ is the only compound in the series which shows a complex low temperature spectrum with several conformers present. On warming the sample, the picture became even more complex.

c-L-AlaSar-D-AlaSar₂ (Fig. 4) and c-L-AlaSar-L-AlaSar₂ show identical NMR spectral changes with rising temperature. Conformer A has three sharp N-CH₃ signals and three well resolved CH₃-quartets. B does not here approach A in concentration before the third conformer C appears. C increases to become the only visible conformation (> 90 %) at room temperature. This indicates that the conformational transformations of these molecules go through the same ring conformations, and that the C₃-methyl groups derived from the D-terminal alanine (D or L) have coincident chemical shifts. Both c-GlySarGlySar₃ and c-L-Ala-D-Ala-L-AlaSar₃ undergo analogous conformational changes with C as the dominating conformer in the final equilibrium mixture.
Conformer A of c-L-Ala-d-Ala-L-AlaSar, has two sharp $N$-CH$_3$ lines and two resolved CH$_3$-quartets both with $J$ of 18 Hz and conformer C has two narrow split N-CH$_3$ lines and two well resolved CH$_3$-quartets with $J$ of 18 and 14 Hz. The CH$_3$-quartets of c-GlySarGlySar, are not resolved and started to coalesce at ca. $-5$ °C.

c-D-Ala-L-AlaSar-D-AlaSar (Fig. 5) shows at low temperature two N-CH$_3$ lines and two CH$_3$-quartets. In this case no conformational change takes place before at about $-15$ °C when a second conformer B develops. On further warming, A diminishes as B increases in concentration, but no third conformer C appears, and at room temperature pure B is present. This is the only compound in the series where only one conformational transformation is observed. The closely related c-D-Ala-L-Ala-Sar-L-AlaSar shows, however, a different conformational behavior. One major conformation A is present at low temperature. The second conformer B becomes equal to A in concentration before the third conformer C develops at about $-30$ °C. In the equilibrium mixture there is hardly any B while A and C are present in equal amounts. These compounds do not adopt the same ring skeletons nor do the same conformational transformations occur, although the only difference is that one $\alpha$-CH$_3$ group in one is inverted to the other. Steric interaction of the three $\alpha$-methyl groups may stabilise different conformations.

ATTEMPTS TO APPLY NMR METHODS FOR DISTINGUISHING BETWEEN cis AND trans AMIDE IN CYCLIC PEPTIDES

In order to understand the conformational changes it was important to know if cis-trans amide interchange was involved. Some attempts were therefore made to investigate if it might be possible to distinguish between the N-CH$_3$ of cis amide bonds and those of trans in the sarcosine peptides. Several methods have been used to distinguish between cis and trans amides, such as differential solvent shifts, different band width of the N-methyl lines, shifts induced by complexation with paramagnetic metal ion and shift differences in $^{13}$C NMR. These effects have been discussed for simple amides and small peptides. The N-methyl groups in N,N-dimethylformamide and related compounds differ in chemical shift, being highest for the methyl group cis to the carbonyl due to the anisotropy of this group. A similar difference is observed in N-acetylsarcosine methyl ester and in N-acetyl-N-methyl-L-alanine methyl ester where the N-methyl protons of the preferred trans conformation are shifted to lower field than the cis N-methyl protons. This assignment has been extended to polysarcosine where the conclusion is drawn that both cis and trans amides are present and to helical poly-N-methyl-L-alanine which shows only trans amides. In an aromatic solvent, like benzene, the N-methyl peaks are shifted upfield, but to different extents due to a specific solvent/solute interaction. The N-methyl trans to the carbonyl shows a greater upfield shift than that cis to the oxygen. This effect appears to be general for simple amide systems as it is observed in several N,N-di-alkylamides, some small, medium and large sized N-methylactams and in small peptides such as N-acetyl-N-methyl-L-alanine methyl ester. In addition to chemical shift differences a broadening of the N-methyl peaks cis to the carbonyl is observed due to a small long range spin coupling through the amide bond to the C$_a$-protons. This coupling is largest when the N-methyl group is anti to a C$_a$-proton. In a series of dipeptides with N-methylated amino acid as the second residue the N-methyl peak which belongs to cis amides is broader than that of trans amides. The different effects described for simple flexible systems were applied to our cyclic sarcosine peptides which differ firstly in being relatively small rings with several amide groups present and secondly in being rigid systems. The most suitable molecule with which to check these techniques was cyclo-tetrasarcosyl where the NMR parameters are known. Cyclo-tetrasarcosyl is a centrosymmetric molecule with the amide sequence alternately cis and trans. Replacement of one sarcosine residue with alanine showed that the upfield N-methyl peak belongs to a trans amide group in contrast to what is usually found in the simple amide systems. Furthermore, the upfield peak of the N-methyl trans to the carbonyl is significantly broader and exhibits a small coupling of about 0.5 Hz. Spin decoupling showed that this is
Table 1. Yields (%) of cyclic peptides from pentapeptide trichlorophenyl ester.

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Dipeptides</th>
<th>Tetrapeptides</th>
<th>Pentapeptides</th>
<th>Decapeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>HClH-AlaSar_OTep</td>
<td>c-Sar(_2) (20 %)</td>
<td></td>
<td>c-AlaSar(_3) (46 %)</td>
<td>c-(AlaSar)(_4) (3 %)</td>
</tr>
<tr>
<td>HClH-Sar_D-Ala_L-AlaSar_OTep</td>
<td></td>
<td></td>
<td>c-D-Ala-L-AlaSar(_4) (38 %)</td>
<td>c-(D-Ala-L-AlaSar)(_5) (traces)</td>
</tr>
<tr>
<td>HClH-Sar(_2)-L-Ala_OTep</td>
<td></td>
<td></td>
<td>c-L-AlaSar(_3) (36 %)</td>
<td>c-(L-AlaSar)(_4) (traces)</td>
</tr>
<tr>
<td>HClH-Sar(_2)-L-AlaSar-D-Ala_OTep</td>
<td>c-Sar(_2) (10 %)</td>
<td>c-L-AlaSar-D-AlaSar(_2) (11 %)</td>
<td>c-L-AlaSar-L-AlaSar(_3) (6 %)</td>
<td>c-(L-Ala-D-Ala-L-AlaSar)(_4) (traces)</td>
</tr>
<tr>
<td>HClH-L-Ala_D-Ala_L-AlaSar_OTep</td>
<td></td>
<td></td>
<td>c-L-AlaSar-D-AlaSar(_2) (11 %)</td>
<td>c-(L-Ala-D-Ala-L-AlaSar)(_5) (traces)</td>
</tr>
<tr>
<td>HClH-Sar(_2)-D-Ala_L-AlaSar_OTep</td>
<td></td>
<td></td>
<td>c-D-Ala-L-AlaSar(_3) (55 %)</td>
<td>c-(D-Ala-L-AlaSar-L-AlaSar)(_4) (5 %)</td>
</tr>
<tr>
<td>HClH-GlySar_OTep</td>
<td></td>
<td></td>
<td>c-GlySar(_3) (53 %)</td>
<td>c-(GlySar)(_4) (traces)</td>
</tr>
<tr>
<td>HClH-SarGlySar_OTep</td>
<td></td>
<td></td>
<td>c-GlySar(_3) (6 %)</td>
<td></td>
</tr>
<tr>
<td>HClH-GlySarGlySar_OTep</td>
<td>c-GlySar (8 %)</td>
<td></td>
<td>c-GlySarGlySar(_2) (4 %)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The chemical shifts of the NCH\(_3\) protons of cyclic peptides in CDCl\(_3\).

<table>
<thead>
<tr>
<th></th>
<th>(NCH(_3))(_1)</th>
<th>(NCH(_3))(_2)</th>
<th>(NCH(_3))(_3)</th>
<th>(NCH(_3))(_4)</th>
<th>(NCH(_3))(_5)</th>
<th>(NCH(_3))(_6)</th>
<th>ml CD(_3)D added to 0.5 ml CDCl(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Sar(_2)</td>
<td>3.0 (19) cis</td>
<td>3.0 (19) cis</td>
<td>2.83 (36) trans</td>
<td>2.83 (36) trans</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>c-Sar(_3)</td>
<td>3.14 (49)</td>
<td>3.01 (36)</td>
<td>2.93 (4)</td>
<td>2.93 (2)</td>
<td>2.91 (18)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>c-Sar(_4)</td>
<td>2.96 (20)</td>
<td>2.96 (35)</td>
<td>2.91 (10)</td>
<td>2.91 (31)</td>
<td>0.35</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>c-Sar(_5)</td>
<td>2.98 (30)</td>
<td>2.92 (13)</td>
<td>2.83 (21)</td>
<td>2.83 (38)</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>c-AlaSar(_2)(B)</td>
<td>3.30 (58)</td>
<td>3.25 (49)</td>
<td>3.12 (57)</td>
<td>3.12 (57)</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>c-AlaSar(_2)(C)</td>
<td>3.18 (80)</td>
<td>3.07 (105)</td>
<td>2.92 (23)</td>
<td>2.92 (23)</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>c-D-Ala-L-AlaSar(_3) (C)</td>
<td>3.20 (52)</td>
<td>3.15 (30)</td>
<td>3.07 (47)</td>
<td>3.07 (47)</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>c-D-Ala-L-AlaSar(_3) (D)</td>
<td>3.17 (41)</td>
<td>3.10 (27)</td>
<td>3.04 (16)</td>
<td>3.04 (16)</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>c-L-AlaSar-D-AlaSar(_2) (C)</td>
<td>3.25 (27)</td>
<td>3.16 (54)</td>
<td>3.11 (27)</td>
<td>3.11 (27)</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>c-L-Ala-D-Ala-L-AlaSar(_3) (C)</td>
<td>3.28 (51)</td>
<td>3.11 (64)</td>
<td>2.85 (22)</td>
<td>2.85 (22)</td>
<td>0.9</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>c-D-Ala-L-AlaSar-D-AlaSar(_2) (B)</td>
<td>3.2 (68)</td>
<td>3.09 (90)</td>
<td>2.89 (26)</td>
<td>2.89 (26)</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>c-GlySar(_2) (C)</td>
<td>3.10 (59)</td>
<td>3.09 (90)</td>
<td>2.81 (35)</td>
<td>2.81 (35)</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) \(\delta\) represents the chemical shift differences in Hz when the given quantity of CD\(_3\)D is added in chloroform. The letters B, C and D represent the different conformations formed from the crystal conformation. OGI stands for the glycolic acid residue, —OCH\(_2\)CO—.
due to coupling to one of the Cα-protons adjacent to the nitrogen, that one which is anti to the N-methyl group (across a w-path). No coupling through the amide bonds was observed. The structure 7 shows none of the Cα-protons across the amide bonds to be anti to the N-methyl groups which had been observed to give the largest coupling constant.8 Drop-wise addition of benzene moved the peaks up-field, the trans N-methyl more than the cis (Table 2), which is in accord with the results from the simple amides. These effects were further checked using the higher cyclic sarcosine homologues with known conformation. Cyclo-pentasarcosyl which contains two trans and three cis amide bonds shows in chloroform solution two "low field" N-methyl peaks and three at higher field. On addition of benzene the former moved upfield to a greater extent than the latter indicating that these low field N-methyl peaks belong to the two trans amides. Of the other three signals two are virtually unaffected by the benzene addition and one shifts slightly upfield (Table 2). Cyclo-octasarcosyl with the amide sequence cis,cis,trans,trans, cis,cis,trans,trans, cis,cis,trans,trans and two-fold symmetry in the molecule has two different trans and two different cis N-methyl groups. In the NMR spectrum in chloroform solution two N-methyl peaks are resolved, but on addition of benzene four peaks appear. One of these is shifted slightly upfield, one significantly upfield, and the remaining two are intermediate. The same situation was found in the related depsipeptide, c-SarOGiSarOGi, which takes the same conformation but with one trans N-methyl peak lacking (Table 2). Peak broadening was difficult to observe in the larger molecules mostly due to the complexity of the spectra. This shows that solvent effects which seem to be general for open chain peptides cannot be extended to cyclic peptides. The folding of the chain into a ring may lead to situations where the chemical shift of an N-methyl group is influenced also by carbonyl groups further along the chain but close in space.

Lanthanide shift reagents which induce pseudocontact shifts by interaction with the lone pair of the carbonyl oxygen, usually shift a methyl group cis to the carbonyl more downfield than one being trans.90 When Eu(fod), was added to a chloroform solution of cyclo-tetrasarcosyl, the cis N-methyl group was shifted more than trans, but in cyclo-pentasarcosyl one of the two "low-field" N-methyl signals assigned to trans, showed the largest shift together with one of the three high-field signals, assigned to cis. The other signals were also shifted, but to a lesser extent. These downfield shifts are not consistent with the upfield shifts found in benzene, but as the Eu-complex, when situated at a given carbonyl group, will to a greater extent than benzene influence all protons of the molecule, it will be difficult to predict the different shifts when several amide groups are involved.

Carbon-13 magnetic resonance has been used to distinguish between cis and trans amides in peptides where the nitrogen of proline is part of the peptide bond.91 Distinct differences in chemical shifts especially for the γ-carbon in the proline ring were found such that the minor cis isomer was shifted about 2 ppm upfield relative to the trans isomer and that the chemical shifts were the same for several compounds of this type. 13C resonance spectra of cyclo-tetrasarcosyl, cyclo-alanyltrisarcosyl and cyclo-pentasarcosyl, for which the conformations are known, and of cyclo-glycyltetrasarcosyl were recorded at 15 MHz in chloroform solution. Cyclo-tetrasarcosyl showed cis and trans peaks, separated by 2—4 ppm for each of the three types of carbon atoms. Cyclo-alanyltrisarcosyl showed four carbonyl carbons where two belong to cis amide bonds and two to trans, but these did not form two distinct groups, the N-CH3 cis and trans were separated by only 1 ppm. In cyclo-pentasarcosyl four of the five carbonyl carbons were resolved, but again not in two groups and the N-CH3's were hardly resolved. These preliminary 13C investigations showed no clear differences in the chemical shifts due to cis and trans amide bonds. The conformationally unknown cyclo-glycyltetrasarcosyl showed a similar picture with no well separated cis-trans peaks.

Thus, none of the different NMR methods used to distinguish between cis and trans amide bonds in small linear peptides showed convincing results when applied to the cyclic homologues of sarcosine and derivatives. Although the benzene addition showed some evidence that the N-CH3 of a trans amide group

moves more upfield than \( N\text{-CH}_3 \) of a \textit{cis} amide group, the difference in values can be small and cannot be relied upon diagnostically. Benzene addition to the cyclic pentapeptides was, however, useful to resolve overlapping signals since the conformation does not change on changing solvent. Table 2 shows the chemical shifts of the \( N\)-methyl protons in CDCl\(_3\) solution and the upfield \( \delta \) shifts when C\(_6\)D\(_4\) is added mainly for the conformations B and C (D). Addition of benzene was also attempted at low temperature to the A-conformation but with little success due to the difficulty in keeping the temperature low enough so that the B and C conformers did not develop.

**DISCUSSION**

The NMR spectra clearly show that in these cyclic pentapeptides the crystal conformations are not the same as those favoured in solution. The conformational transformations might well go through additional transient unpopulated conformers not visible in the NMR spectra, but this seems hardly likely and will not be further considered. Conformational transformations seem to be prominent for cyclic pentapeptides containing NH-amide groups. Only the parent compound, c-Sar\(_4\), retains the same conformation in solution as in the crystal.\(^6\) Replacement of one or more sarcosine units with NH-amino acids as glycine or alanine lead to a mixture of conformations in solution with the crystal conformation hardly present. The ability of the NH-amides to form hydrogen bonds has already been proposed as the driving force for these conformational changes.\(^8\) External hydrogen bonds stabilise the crystal conformers while intramolecular hydrogen bonds stabilise the conformers in solution, and the conformational changes must involve stepwise transformations of \textit{cis} amide bonds to \textit{trans}. Among the NH-containing cyclic pentapeptides only c-AlaSar\(_4\)\(^{18}\) (Fig. 6A) has had its crystal conformation determined by X-ray methods. It is found to be identical to that of c-Sar\(_4\)\(^{18}\) both having the amide sequence \textit{cis, cis, cis, trans, trans}(NH). The NH-amide group which occupies one of the two \textit{trans} positions makes an external hydrogen bond with the carbonyl oxygen of the other \textit{trans} amide group of a neighbouring molecule. On dissolution, the intermolecular hydrogen bonds are lost and the new conformer B may be favoured by a hydrogen bond across the ring. This is only possible if one of the \textit{cis} amide groups becomes \textit{trans}, and the sequence \textit{cis, cis, trans, trans, trans}(NH) is suggested\(^3\) (Fig. 6B). The more stable conformer C may arise by transformation of yet another \textit{cis} amide bond to \textit{trans}, to the sequence \textit{trans, cis, trans, trans, trans}(NH) (Fig. 6C). Both B and C resemble the conformations found by X-ray studies of c-Gly\(_4\)\(^{32}\) and c-Ala\(_3\)Gly\(_4\)\(^{34}\) which contain both inter- and intramolecular hydrogen bonds.

It is likely that all cyclic pentapeptides which can easily fit the crystal conformation of c-AlaSar\(_4\), without having any NH-amide group \textit{cis}, will adopt this skeleton in the crystal. Thus, c-GlySar\(_4\) may take this skeleton with the NH-amide in one of the two \textit{trans} positions, but not necessarily the same position as in c-AlaSar\(_4\) since the \( \alpha \)-substituent is now lacking. The same conformational transformations may take place, but in this case conformer B is less stable compared to C as the mixture at equilibrium hardly contains any.

\[ R = \text{H or Me} \]

**Fig. 6.**

B. Both c-D-Ala-L-AlaSar₄ and c-Gly₃Sar₂ fit the crystal skeleton of c-AlaSar₄ with the two NH-amides in the trans positions, and the same conformers B and C can be formed also here. The last transformation to D seems less clear but may well correspond to the conversion of the remaining cis amide bond to trans, giving a conformation with all amide bonds trans as the most stable. Two intramolecular hydrogen bonds can then be formed as shown in Fig. 6D which is just one example among several possibilities for internal hydrogen bonding.

c-L-AlaSar-D-AlaSar₄ and c-L-AlaSar-L-AlaSar₄ which have identical NMR spectra both at low and high temperatures cannot easily adopt the crystal conformation found for c-AlaSar₄ which would require one NH-amide bond to be cis. A crystal structure of a related molecule, actinomycin, which has two identical cyclic pentapeptides, (16-membered ring) shows the amide sequence cis,cis,trans,trans,trans with the nitrogens of proline and sarcosine as part of the cis amide bonds and a strong hydrogen bond between the pentapeptides.⁵² Two adjacent cis amide bonds are also found in some sarcosine peptides and it seems therefore likely that the crystal conformation of c-L-AlaSar-D-AlaSar₄ has the amide sequence cis,cis,trans (NH), trans,trans (NH), the same sequence as suggested for conformer B of c-AlaSar₄, and with one intra- and one intermolecular hydrogen bond. The conformational transformations may occur as for c-AlaSar₄ where the conformer C (Fig. 6C) corresponds to conformer B in c-L-AlaSar-D-AlaSar₄ with the amide sequence trans,cis,trans (NH), trans,trans (NH) and one intramolecular hydrogen bond. The second transformation leads to conformer C which should have all the amide bonds trans and be similar to that in Fig. 6D. c-GlySarGlySar, and c-L-Ala-D-Ala-L-AlaSar₄ can go through the same conformational transformations and take the same ring conformations without ever having any NH amide bond in cis, and the NMR spectra which show closely related changes support this picture.

c-D-Ala-L-AlaSar-D-AlaSar which undergoes only one conformational change from A to B (Fig. 5) cannot easily adopt any of the two aforementioned skeletons for the crystal conformation, but is believed to have a ring conformation close to that in Fig. 6C with the amide sequence cis,trans (NH), trans (NH), trans, trans (NH). The transformation of conformer A to B may then correspond to a conversion of the remaining cis amide bond to trans (Fig. 6D). c-D-Ala-L-AlaSar-D-AlaSar which shows two conformational changes may have the amide sequence cis,trans (NH), trans (NH), cis, trans (NH) for conformer A. B may originate by transformation of one of the two cis amide bonds to trans, giving the sequence trans,trans (NH), trans (NH), cis,trans,trans (NH) and further transformation of the last cis amide bond leading to conformer C.

In this series of cyclic pentapeptides trans seems to be the most stable in chloroform solution as the number of observed transformations corresponds to the initial number of cis amide bonds present. The only exceptions are c-AlaSar₄ and c-GlySar₄ which can form only one intramolecular hydrogen bond. Of course, these arguments imply that the barrier for each of the successive transformations is higher than the preceding one.

More definite conclusions about these conformational changes and the nature of the different conformers which are formed must await further detailed NMR studies (¹H) and more X-ray structural studies.

EXPERIMENTAL

Solvents used in the reactions were of analytical grade, the light petroleum had b.p. 40 - 60 °C. The monobeded ion-exchange resin, Amberlite, MB-1, analytical grade, was used, and the eluent was methanol. The fully protected linear peptides could all be prepared in methylene chloride. After removal of the benzyloxy-carbonyl group was the residual peptide ester (confirmed by NMR) used immediately on isolation.

Many of the peptide derivatives were either viscous oils or non-crystalline solids. Their identity and purity were confirmed by NMR spectroscopy (Varian 60 A) and thin-layer chromatography performed on silica gel G in various solvent systems: ethyl acetate — chloroform 1:2 (A), 5 % methanol — chloroform (B), 10 % methanol — chloroform (C), 15 % methanol — chloroform (D), 20 % methanol — chloroform (E), acetic acid — methanol — chloroform 2:8:90 (F), acetic acid — methanol — chloroform 5:15:80 (G), acetic acid — water — ethanol 10:10:80 (H).

Samples for elemental analysis of the Z-peptide esters were purified on a silica gel column eluting with chloroform, otherwise stated. For the pentapeptides the chloroform elution was followed by chloroform added 2% methanol, and for the cyclic peptides 2–10% methanol was added to the chloroform. The tendency for the cyclic pentapeptides to contain water even after extensive drying as shown by NMR spectroscopy rendered elemental analysis relatively uninformative.

Abbreviations: Et₂N = triethylamine, DCC = dicyclohexylcarbodiimide, HATU = 2,4,6-trichlorophenol, DMF = N,N-dimethylformamide.

**Cyclo-alanyl tetrasarcosyl**

General method for preparation of benzyl-oxycarbonyl-peptide ester (Z-Sar₄-OMe).³

Z₁-AlaSar₄-OMe. Sarcosine methyl ester hydrochloride ³ (55 g = 250 mmol) was suspended in CH₂Cl₂ (500 ml), Et₂N (25.5 g = 250 mmol) and added the solution filtered before combined with benzylxycarbonyl-L-alanine ³⁷ (56 g = 250 mmol) in CH₂Cl₂ (100 ml), cooled to −15 °C and DCC (53.5 g = 260 mmol) added in portions. The reaction mixture was warmed to attain room temperature during 15 h and worked up as described.⁴ The residual oil was dissolved in ether and precipitated with light petroleum to leave a viscous oil (72 g = 93 %), homogeneous by TLC, Rf 0.5 (A).

General method for preparation of the benzylxycarbonyl-peptide acids (Z-Sar₄-OH).⁴

Z₁-AlaSar₄-OH. Z₁-AlaSar₄-OMe (70 g = 227 mmol) was hydrolysed in 1 N NaOH (230 ml) for 2 h and the mixture concentrated to ca. 40 ml and extracted twice with CH₂Cl₂. The aqueous layer was then acidified, extracted with CH₂Cl₂, washed with water and dried. The resultant oil was treated with ether and light petroleum and crystallised from acetone on addition of ether (56 g = 84 %). TLC, Rf 0.5 (F), m.p. 137 °C, [α]D²⁵ −28.3° (c 2, MeOH). (Found: C 57.17; H 6.33; N 9.66. C₁₄H₁₆N₂O₄ requires C 57.13; H 6.17; N 9.52).

Z₁-AlaSar₄-OMe. Z₁-AlaSar₄-OH (6.5 g = 22 mmol), H-Sar₄-OMe ⁴ (5.3 g = 21.6 mmol) and DCC (5 g = 24.2 mmol) in CH₂Cl₂ (100 ml) resulted in a white solid (0 g = 80 %). TLC, Rf 0.45 (C), m.p. 65 °C, [α]D²⁵ −5.7° (c 3, MeOH). (Found: C 54.95; H 6.75; N 13.46. C₁₄H₂₂N₂O₄ requires C 55.26; H 6.76; N 13.43). Z₁-AlaSar₄-OH. Z₁-AlaSar₄-OMe (7.8 g = 15 mmol) and NaOH (16 ml) was reacted for 2 h. After acidification the acid was extracted into chloroform, dried and evaporated. The resulting peptide acid (7 g) was purified by extraction from a CH₂Cl₂ solution into an alkaline aqueous layer, acidification with 2 N hydrochloric acid and extraction into chloroform, followed by drying and evaporation. This afforded a white powder (6.7 g = 88 %), TLC, Rf 0.45 (G), softening at 95 °C, [α]D²⁵ −5.9° (c 2, MeOH). (Found: C 54.42; H 6.49; N 13.76. C₁₂H₁₄N₂O₄ requires C 54.43; H 6.55; N 13.80).

General method for preparation of benzyl-oxycarbonyl-peptide 2,4,6-trichlorophenyl ester (Z-Sar₄-OTcp).⁶

Z₁-AlaSar₄-OTcp. Z₁-AlaSar₄-OH (3.7 g = 7.3 mmol), HATU (2.2 g = 11.1 mmol) and DCC (1.6 g = 7.7 mmol) in CH₂Cl₂ (50 ml) gave a white solid (4.5 g = 90 %), TLC, Rf 0.6 (C). An analytical sample (0.3 g) was further purified on a small silica gel column, softened at 75 °C. (Found: C 50.98; H 5.08. C₁₃H₁₄N₂O₄Cl requires C 50.70; H 4.99).

General method for preparation of cyclic peptides (ε-Sar₄).

**Cyclisation.** Z₁-AlaSar₄-OTcp (1 g = 1.4 mmol) was hydrogenated for 50 min in methanol (80 ml) containing conc. HCl (0.1 ml) and 5 % Pd·C (0.3 g). The resulting yellowish semi solid (0.8 g = 1.36 mmol = 84 %) of HCl·Z₁-AlaSar₄-OTcp was dissolved in DMF (50 ml) and added dropwise to stirred pyridine (500 ml) at 115 °C over a period of 1 h and stirred for another 1.5 h. After evaporation the residue was dissolved in methanol (100 ml), passed through a ion-exchange column and eluted with methanol. The first eluate (200 ml) contained most of the cyclic peptides. This was evaporated to dryness, treated with acetone (5 ml) and the undissolved material cyclo-alanyl tetrasarcosyl filtered off. The filtrate was combined with the remaining column eluate (400–600 ml), which contained further cyclic peptides together with some DC-urea, this total solution evaporated to dryness and purified on a silica gel column, eluting with chloroform followed by chloroform-methanol (2–10 %). Yields of cyclic compounds: Cyclo-alanyl tetrasarcosyl (45.5 %), TLC, Rf 0.45 (D), m.p. 245–250 °C, m/e 583, [α]D²⁵ −2.3° (c 2, MeOH), cyclo-disarcosyl (20 %), cyclo-alanyl sarcosyl (14 %), cyclo-alanlytetrasarcosyl-cyclo-alanyl tetrasarcosyl (3 %), m.p. 195 °C, m/e 710. The cyclo-alanlytetrasarcosylalanyl tetrasarcosyl was not isolable on the column but was isolated by precipitation from methanol-ether.

**Cyclo-alanylalanylalanyltri-sarcosyl**

Z-Sar₄-Ala-OMe. D-Alanine methyl ester hydrochloride ³⁸ (14 g = 100 mmol), Et₂N (10.5 g = 104 mmol), benzylxycarbonylisarcosine (22.7 g = 102 mmol) and DCC (22 g = 107 mmol) gave a semi solid (27 g = 87 %), TLC, Rf 0.5 (A). A sample was purified. (Found: C 58.63; H 6.67; N 8.90. C₁₃H₁₄N₂O₄ requires C 58.43; H 6.54; N 8.09). Z-Sar₄-Ala-OH. Z-Sar₄-Ala-OMe (13 g = 42.2 mmol) and NaOH (43 ml) (1.5 h) gave a solid, recrystallised from acetone (10.6 g = 86 %), TLC, Rf 0.55 (F), m.p. 115–118 °C, [α]D²⁵ −6.1° (c 2, MeOH). (Found: C 57.15;

Z-L-Ala-Sar₄-O-Me. Sarcosine methyl ester hydrochloride (15 g = 108 mmol), Et₃N (11 g = 109 mmol), Z-L-Ala-Sar-OH (30 g = 102 mmol) and DDC (23 g = 110 mmol) gave an oil (34 g = 88 %), TLC, R₅ 0.5 (C).

Z-Sar-L-Ala-L-Ala-Sar₄-O-Me. Z-Sar-L-Ala-OH (10 g = 34 mmol), H-L-Ala-Sar₄-O-Me (8.5 g = 34.7 mmol) [obtained by hydrogenation of Z-L-Ala-Sar₄-O-Me (13.5 g = 35.6 mmol)], DCC (7.7 g = 37.4 mmol) resulted in a foamy solid (15.2 g = 86 %), TLC, R₅ 0.5 (C). An analytical sample softened at 70 °C, [α]D⁺²⁺ + 18.2° (c 2, MeOH). (Found: C 55.05; H 6.83; N 13.60. C₁₄H₁₄N₄O₄ requires C 55.26; H 6.76; N 13.43.)

Z-Sar-L-Ala-L-Ala-Sar₄-O-Me. Z-Sar-L-Ala-Sar₄-O-Me (11 g = 21 mmol) and NaOH (22 ml) (2 h) resulted in a solid, recrystallised from acetone (9.3 g = 87 %), TLC, R₅ 0.5 (G), softened at 90 °C, [α]D⁺²⁺+ 5° (c 2, MeOH). (Found: C 54.37; H 6.50; N 14.08. C₁₄H₁₄N₄O₄ requires C 54.43; H 6.55; N 13.80.)

Z-Sar-L-Ala-L-Ala-Sar₄-O-Me. Z-Sar-L-Ala-OH (5 g = 9.86 mmol), HΟTCP (2.8 g = 14.2 mmol) and DCC (2.2 g = 10.7 mmol) gave a semi-solid (5.4 g = 80 %), TLC, R₅ 0.55 (C). An analytical sample softened at 70 °C. (Found: C 50.76; H 5.22. C₁₄H₁₄N₄O₄Cl requires C 50.70; H 4.99.)

Cyclisation. Z-Sar-L-Ala-L-Ala-Sar₄-O TCP (1.2 g = 1.75 mmol) was hydrogenated and the white solid (0.9 g = 1.55 mmol = 88 %) of HCl-H-Sar₄-L-Ala-Sar₄-O TCP cyclised and worked up as described earlier. The residue after passage through an ion-exchange column was taken into small amount of acetone and undissolved material filtered off; a further precipitate formed when kept at 0 °C overnight. The solid consisted mainly of cyclo-d-di-α-l-Ala₄-l-Ala₄-O TCP cyclised and passed through a small silica gel column. Yield of cyclic compound: Cyclo-di-L-alanyltriarcosyl (38 %), TLC, R₅ 0.45 (D), m.p. 270 °C (subl.), m/e 355, [α]D⁺²⁺ + 14° (c 1, MeOH).

Cyclo-d-i-L-alanyltriarcosyl

Z-Sar₄-L-Ala₄-O'Bu. Z-L-Ala₄-O'Bu (12 g = 34.5 mmol) (prepared from Z-L-Ala-OH and H-L-Ala-O'Bu [using DCC]) was hydrogenated and the oil of H-L-Ala-O'Bu (7 g = 32.4 mmol) reduced with Z-Sar₄-O'H * (9.5 g = 52.3 mmol) and DCC (7.0 g = 34 mmol) in CH₂Cl₂ (300 ml) resulted in a solid (13 g = 81 %), TLC, R₅ 0.6 (C), purified m.p. 90 °C, [α]D⁺²⁺ - 52° (c 2, MeOH).

Z-Sar₄-L-Ala₄-O'Bu. Z-Sar₄-OH (1.8 g = 8.1 mmol), H-Sar₄-L-Ala₄-O'Bu (2.9 g = 8.1 mmol) [obtained by hydrogenation of Z-L-Ala₄-L-Ala₄-O'Bu (4 g = 8.15 mmol)] and DCC (1.8 g = 8.7 mmol) in CH₂Cl₂ (50 ml) gave a white solid (4 g = 88 %), TLC, R₅ 0.35 (C). An analytical sample softened at 70 °C, [α]D⁺²⁺ - 47.6° (c 2, MeOH). (Found: C 57.39; H 7.32; N 12.46. C₁₄H₁₄N₄O₄ requires C 57.53; H 7.33; N 12.43.)

Z-Sar₄-L-Ala₄-OH. Z-Sar₄-L-Ala₄-O'Bu (3.6 g = 8.4 mmol) was dissolved in ice-cooled trifluoroacetic acid (20 ml), stirred at room temperature for 40 min, evaporated and poured into ether. The ether was decanted, the solid dissolved in acetone and precipitated with ether (2.6 g = 80 %), TLC, R₅ 0.4 (G), softened at 85 °C. (Found: C 54.65; H 6.53. C₁₄H₁₄N₄O₄ requires C 54.43; H 6.55.)

Z-Sar₄-L-Ala₄-OTCP. Z-Sar₄-L-Ala₄-OH (1.7 g = 3.55 mmol), HΟTCP (1.2 g = 6.1 mmol) and DCC (0.86 g = 4.1 mmol) resulted in a semi-solid (2 g = 57 %), TLC, R₅ 0.65 (C). A sample for analysis was crystallised from acetone-ether, softened at 80 °C. (Found: C 50.97; H 5.62; N 10.16. C₁₄H₁₄N₄O₄Cl requires C 50.70; H 4.99; N 10.19.)

Cyclisation. Z-Sar₄-L-Ala₄-O TCP (1.9 g = 2.77 mmol) was hydrogenated and the HCl-H-Sar₄-L-Ala₄-O TCP (1.5 g = 25.6 mmol = 93 %) cyclised. The residue after passage through an ion-exchange column was taken into water to remove undissolved DC-urea and the evaporated residue which showed one main spot on TLC belonging to a cyclic pentapeptide and one minor spot which presumably belongs to the corresponding cyclic decapeptide was chromatographed on a silica gel column. Yield of cyclic compound: Cyclo-di-L-alanyltriarcosyl (35.4 %), TLC, R₅ 0.45 (D), m.p. 225–230 °C, m/e 365, [α]D⁺²⁺ + 20° (c 1, MeOH).

Cyclo-L-alanylsercosyl-d-alanyldisarcosyl

Z-L-Ala-Sar₄-D-Ala-O'Me. D-Alanine methyl ester hydrochloride (5.8 g = 41.7 mmol), Et₃N (4.3 g = 42.5 mmol), Z-L-Ala-Sar-OH (12 g = 41 mmol) and DCC (9 g = 43.5 mmol) gave a viscous oil (13 g = 84 %), TLC, R₅ 0.6 (C). A sample was purified. (Found: C 57.15; H 6.64; N 10.99. C₁₄H₁₄N₄O₄ requires C 56.98; H 6.64; N 11.08.)

Z-Sar₄-L-Ala-Sar₄-D-Ala-O'Me. Z-Sar₄-OH (5 g = 17 mmol), H-L-Ala-Sar₄-D-Ala-O'Me (4.2 g = 17.1 mmol) [obtained by hydrogenation of Z-L-Ala-Sar₄-D-Ala-O'Me (6.5 g = 17.2 mmol)], DCC (3.9 g = 18.9 mmol) gave a foamy solid (7.2 g = 81 %) which on further purification was homogeneous by TLC, R₅ 0.4 (C) softened at 65 °C, [α]D⁺²⁺ + 5.6° (c 2, MeOH).

Cyclo-I-alanyl-D-alanyl-L-alanyl disarcosyl

Z-L-Ala-D-Ala-L-Ala-Sar-O-Me. Z-L-Ala-D-Ala-OMe (6.2 g = 21.1 mmol) (prepared from Z-L-Ala-OMe and HCl.-H-D-Ala-OMe using DCC, the protected dipeptide methyl ester was hydrolysed), H-L-Ala-Sar-OMe (5.8 g = 23.6 mmol) (obtained by hydrogenation of Z-L-Ala-Sar-OMe (9.3 g = 24.6 mmol)) and DCC (4.8 g = 23.3 mmHg) in CHCl₃ (70 ml) resulted in a foamy solid (9 g = 82 %), TLC, RF 0.45 (C). An analytical sample softened at 80°C, [α]D₂⁰ +3.2° (c 2, MeOH). (Found: C 55.25; H 6.66; N 13.38. C₁₈H₂₆N₄O₅ requires C 55.26; H 6.76; N 13.43).

Z-L-Ala-D-Ala-L-Ala-Sar-OH. Z-L-Ala-D-Ala-L-Ala-Sar-O-Me (7.2 g = 13.8 mmol) in NaOH (15 ml) (2 h) gave a solid which was crystallised from acetic acid—ether (65 g = 93 %), TLC, RF 0.4 (G), softened at 85°C, [α]D₂⁰ +1° (c 2, MeOH). (Found: C 54.60; H 6.65; N 13.92. C₁₈H₂₆N₄O₅ requires C 54.43; H 6.55; N 13.80).

Z-L-Ala-D-Ala-L-Ala-Sar-OTCP. Z-L-Ala-D-Ala-L-Ala-Sar-OH (3 g = 5.9 mmol), HOTerp (1.7 g = 8.6 mmol) and DCC (1.35 g = 6.55 mmol) resulted in a white powder (3.6 g = 89 %), TLC, RF 0.4 (C), softened at 85°C.

Cyclo-I-Ala-D-Ala-L-Ala-Sar-OTCP (0.9 g = 1.3 mmol) was hydrogenated and the HCl·H-L-Ala-D-Ala-L-Ala-Sar-OTCP (0.7 g = 1.2 mmol = 91 %) cyclised. The residue after passage through an ion-exchange column was dissolved in acetone (5 ml) and kept overnight at 0°C. The precipitate contained only a few mg of the cyclic pentamer and some DC-urea which was removed by sublimation, to 200°C at reduced pressure. The filtrate was evaporated and purified on a silica gel column to give a white compound homogeneous by TLC, but which melted over a wide range. The solid became crystalline when dissolved in acetone (2 ml) and allowed to evaporate to dryness by keeping the sample open in a refrigerator. The residue, cyclo-I-Ala-D-Ala-L-Ala-Sar-disarcosyl, was washed carefully with acetone and the white powder isolated (28 %) possessed a relatively sharp melting point, m.p. 208–210°C, TLC RF 0.6 (D), m/e 355, [α]D₂⁰ +0° (c 2, MeOH).

Cyclo-D-alanyl-L-alanyl disarcosyl-D-alanyl disarcosyl

Z-Sar-D-Ala-L-Ala-Sar-D-Ala-O-Me. Z-Sar-D-Ala-OMe (5 g = 17 mmol), H-L-Ala-Sar-D-Ala-OMe (4.2 g = 17.1 mmol) (obtained by hydrogenation of Z-L-Ala-Sar-D-Ala-OMe (6.5 g = 17.2 mmol)) and DCC (4 g = 19.4 mmol) resulted in a foamy solid (7 g = 79 %), TLC, RF 0.45 (C). An analytical sample softened at 50°C, [α]D₂⁰ +39.6° (c 2, MeOH). (Found: C 54.92; H 6.91; N 13.68. C₁₈H₂₆N₄O₅ requires C 55.26; H 6.76; N 13.43).

Z-Star-D-Ala-L-AlaSar-D-Ala-OH. Z-Star-D-Ala-L-AlaSar-D-Ala-OMe (7.3 g = 14 mmol) in NaOH (13 ml) (3 h) gave a solid, recrystallised from acetone–ethanol (6.8 g = 93 %), TLC, Rf 0.5 (G), m.p. 86 °C, [α]D + 36.7 (c 2, MeOH). (Found: C 54.54; H 6.50. C₁₁H₂₁N₄O₄ requires C 54.43; H 6.55). 

Z-Star-D-Ala-L-AlaSar-D-Ala-OTcp. Z-Star-D-Ala-L-AlaSar-D-Ala-OH (4 g = 7.9 mmol), HOTcp (2.3 g = 11.7 mmol) and DCC (1.8 g = 8.7 mmol) gave a solid (4.6 g = 85 %), TLC, Rf 0.7 (C), soften at 75 °C.

Cyclisation. Z-Star-D-Ala-L-AlaSar-D-Ala-OTcp (1.4 g = 2 mmol) was hydrogenated and the HCl-H-Star-D-Ala-L-AlaSar-D-Ala-OTcp (1.1 g = 1.9 mmol = 92 %) cyclised. The powder left after passage through an ion-exchange column consisted of two compounds (I and II) with approximately the same Rf-values (TLC, solvent D). Most of the major compound (I) could be filtered off after suspending the powder in acetone. The filtrate which still contained both compounds was passed through a silica gel column and the isolated compounds (I and II) both had m/e 355 which is the molecular ion required for the cyclic pentapeptide. The major compound (I) is assumed to be cyclic-d-alanyl-L-alanylarginyl-D-alanylalanylarginyl (53 %), TLC, Rf 0.5 (D), m.p. 317 °C (sublim.), m/e 355, [α]D +18 °C (c 2, MeOH). (Found: C 50.88; H 6.92; N 19.87. C₁₁H₁₉N₄O₄ requires C 50.69; H 7.09; N 19.71). The minor compound (II) is assumed to be cyclic-d-alanyl-L-alanylarginyl-L-alanylarginyl (3 %), TLC, Rf 0.05 (D), m.p. 260 °C, m/e 355, [α]D +10 °C (c 0.4, MeOH).

Cyclo-glycyrltetrasarcosyl

Z-GlySar4-OMe. Sarcosine methyl ester hydrochloride (14 g = 100 mmol), Et₂N (10.5 g = 104 mmol), benzylisocyanamide (27 g = 100 mmol) and DCC (22 g = 107 mmol) resulted in a viscous oil (25 g = 85 %), TLC, Rf 0.7 (B). A sample was purified. (Found: C 56.92; H 5.91; N 9.60. C₁₁H₁₉N₄O₄ requires C 57.13; H 6.17; N 9.52).

Z-GlySar-OH. Z-GlySar-OMe (27 g = 92 mmol) in NaOH (93 ml) (24 h) gave a solid, recrystallised from acetone (20 g = 78 %), TLC, Rf 0.5 (F), m.p. 95–97 °C. (Found: C 55.26; H 5.62; N 10.01. C₁₁H₁₉N₄O₄ requires C 55.71; H 5.75; N 10.0).

Z-GlySar-OMe. Z-GlySar-OH (6.5 g = 23.2 mmol), H-Sar₃-OMe * (5.7 g = 23.2 mmol) and DCC (5.2 = 25.2 mmol) gave a foamy solid (9.2 g = 78 %), TLC, Rf 0.3 (C), soften at 70 °C.

Z-GlySar-OH. Z-GlySar-OMe (7.5 g = 14.8 mmol) in NaOH (16 ml) (3 h) gave a solid (6.2 g = 85 %), TLC, Rf 0.6 (H), soften at 80 °C. (Found: C 53.5; H 6.36; N 14.19. C₁₁H₁₉N₄O₄ requires C 53.54; H 6.33; N 14.19).

Z-GlySar-OTcp. Z-GlySar-OH (3 g = 6.1 mmol), HOTcp (2 g = 10 mmol) and DCC (1.5 g = 7.3 mmol) gave a solid, recrystallised from acetone (3.8 g = 90 %), TLC, Rf 0.65 (D), m.p. 135 °C. (Found: C 50.04; H 4.90; N 10.25. C₁₁H₁₉N₄O₄ requires C 49.98; H 4.79; N 10.41).

Cyclisation. Z-GlySar₄-OTcp (2 g = 2.98 mmol) was hydrogenated and the HCl-H-GlySar₄-OTcp (1.6 g = 2.5 mmol = 93 %) cyclised. The residue after passage through an ion-exchange column was suspended in water to remove the DC-urea by filtration, the water was removed from the filtrate and acetone (5 ml) added, some insoluble material was filtered off. This was pure cyclo-glycyrltetrasarcosyl and a further crop crystallised out when the solution was kept in a refrigerator. A small amount of cyclo-glycyrltetrasarcosyl-L-glycyrltetrasarcosyl (TLC) was present in the filtrate but could not be isolated by these methods. Yield of cyclic compound: cyclo-glycyrltetrasarcosyl (52.2 %), TLC, Rf 0.25 (D), Rf 0.3 (E), m.p. 230 °C, m/e 341.

Cyclo-glycyrltetrasarcosyl

Z-GlySar₄-OMe. HCl-H-Star-OMe (7 g = 50 mmol), Et₂N (5.5 g = 54 mmol), Z-GlySar-OH (14 g = 50 mmol) and DCC (11 g = 53 mmol) resulted in an oil (14 g = 77 %), TLC, Rf 0.7 (C) which did not crystallise from acetone on addition of ether. A sample was purified. (Found: C 55.84; H 6.63; N 11.42. C₁₁H₁₉N₄O₄ requires C 55.88; H 6.35; N 11.50).

Z-GlySar₃-OMe. Z-GlySar₄-OMe (10 g = 27.4 mmol) was hydrogenated and the oil of H-GlySar₄-OMe (6.3 g = 27 mmol) dissolved in CH₃CN (200 ml) together with Z-Gly-OH (5.6 g = 27 mmol) and DCC (6.2 g = 30 mmol) and the resulting solid (9 g = 82 %) recrystallised from acetone, TLC, Rf 0.45 (C), m.p. 120 °C. (Found: C 54.81; H 6.59; N 13.11. C₁₁H₁₉N₄O₄ requires C 55.03; H 6.47; N 12.84).

Z-Sar₄-Sar₃-OMe. Z-Sar₄-OH (4.2 g = 18.8 mmol), H-GlySar₄-OMe (5.4 g = 18.8 mmol) [obtained by hydrogenation of Z-GlySar₄-OMe (8 g = 19 mmol)] and DCC (4.3 g = 20.8 mmol) gave a solid (7.9 g = 85 %), TLC, Rf 0.6 (D). An analytical sample softened at 65 °C. (Found: C 53.46; H 6.42; N 13.92. C₁₁H₁₉N₄O₄ requires C 53.54; H 6.33; N 14.19).

Z-Sar₄-Sar₄-OH. Z-Sar₄-Sar₃-OMe (7.2 g = 14.6 mmol) was hydrolysed for 2 h in NaOH (16 ml). Extraction of the acidified aqueous layer with chloroform afforded only 4.5 g of the corresponding acid. In order to isolate more of the acid, the aqueous layer was evaporated nearly to dryness, and extraction with chloroform gave a further 1.8 g (6 g = 86 %), TLC, Rf 0.7 (H), softened at 90 °C.

Z-Sar₄-Sar₄-OTcp. Z-Sar₄-Sar₃-OH (2 g = 14.0 mmol), HOTcp (1.3 g = 6.6 mmol) and DCC (1 g = 4.86 mmol) gave a solid, recrystallised from acetone (2.4 g = 87 %), TLC, Rf 0.7 (D), softened at 90 °C. (Found: C 49.27;

Cyclisation. Z-SarGlySar$_2$-OTcep (1.5 g = 2.28 mmol) was hydrogenated and the HCl.H-SarGlySar$_2$-OTcep (1.2 g = 2.15 mmol = 94%) cyclised. The residue after passage through an ion-exchange column was dissolved in methanol (1 ml) and the solvent allowed to evaporate by keeping the sample open in a refrigerator. The residue was washed carefully with methanol to give a white powder of cyclo-diglycylictrisarcosyl (5.7%), TLC, $R_F$ 0.3 (E), m.p. 209–211 °C, $m/e$ 327.

### Cyclo-glycylsarcosylglycylsarcosyl

Z-GlySarGlySar$_2$-OMe. Z-GlySar-OMe (5 g = 17.8 mmol), H-GlySar$_2$-OMe (4 g = 17.3 mmol) and DCC (4 g = 19.4 mmol) gave a foamy solid (7.4 g = 87%), TLC, $R_F$ 0.3 (C). An analytical sample was obtained at 65 °C. (Found: C 53.42; H 6.36; N 13.91. $C_{12}H_{16}NO_7$ requires C 53.54; H 6.33; N 14.19).

Z-GlySarGlySar$_2$-OH. Z-GlySarGlySar$_2$-OMe (6.4 g = 13 mmol) in NaOH (14 ml) (2½ h) gave the acid (5.5 g = 88%), TLC, $R_F$ 0.65 (H), softened at 80 °C. (Found: C 52.46; H 5.66. $C_{12}H_{14}N_2O_7$ requires C 52.60; H 6.10).

Z-GlySarGlySar$_2$-OTcep. Z-GlySarGlySar$_2$-OH (3 g = 6.3 mmol), HOIOTcep (1.8 g = 9.1 mmol) and DCC (1.5 g = 7.28 mmol) gave a solid, crystalline from acetone; (3.7 g = 90%), TLC, $R_F$ 0.65 (D), m.p. 145–147 °C. (Found: C 49.07; H 4.88. $C_{12}H_{14}N_2O_7$ requires C 49.22; H 4.59).

Cyclisation. Z-GlySarGlySar$_2$-OTcep (1.35 g = 2.05 mmol) was hydrogenated and the HCl.H-SarGlySar$_2$-OTcep (0.9 g = 1.6 mmol = 79%) cyclised. The residue after passage through an ion-exchange column was chromatographed on a silica gel column and two cyclic compounds were isolated, cyclo-glycylsarcosyl (8.3%) and cyclo-glycylsarcosylglycylsarcosyl, the latter melting over a wide range. The cyclic pentapeptide became more crystalline by dissolving in methanol (1 ml) and allowing the solvent to evaporate by keeping the sample open in a refrigerator. The residue was washed carefully with methanol and the white powder (4%) of cyclo-glycylsarcosylglycylsarcosyl was homogeneous by TLC, $R_F$ 0.3 (E), had m/e 327 and melted at 222–230 °C.

**Acknowledgement.** I thank Professor J. Dale who contributed to this work through many helpful discussions especially regarding the conformational aspects. This work was supported by the Norwegian Research Council for Science and Humanities and by Boreggaard Industries Limited, Sarpsborg.

### REFERENCES


Received March 15, 1976.