

Cyclic Peptides of Sarcosine. Syntheses and Conformation

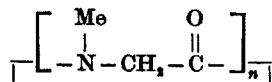
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A series of cyclic peptides of sarcosine with the general formula c-Sar_n, $n=2-8$, has been synthesized and conformational studies carried out both in solution and in the solid. The rings are conformationally very homogeneous and contain both *cis* and *trans* amide bonds. Their barriers to ring inversion are high; in the smaller rings this is attributed to steric hindrance, caused by the *N*-methyl-groups, whilst in the larger rings the folding of the chain in helical segments plays an important role.

Conformational studies have been reported for a large number of cyclic peptides from the simplest dipeptides to more complex biologically active molecules.¹ The majority of these investigations have been concerned with the common primary amino acids where both steric hindrance caused by the α -substituent, and hydrogen bonding, restrict the number of possible conformers, especially in the smaller rings. As we wanted to study cyclic peptides with a greater degree of freedom to take an unperturbed ring conformation, we chose sarcosine as the basic unit. In addition to excluding the aforementioned restrictions, the strong *trans* preference of the *N*-H amides is also nullified and therefore both *cis* and *trans* amide bonds become possible.*

A series of cyclic peptides of sarcosine with the general formula (I) has been synthesized and some results from the conformational studies communicated.²⁻⁴



I, $n=2, 3, 4, 5, 6, 7, 8$

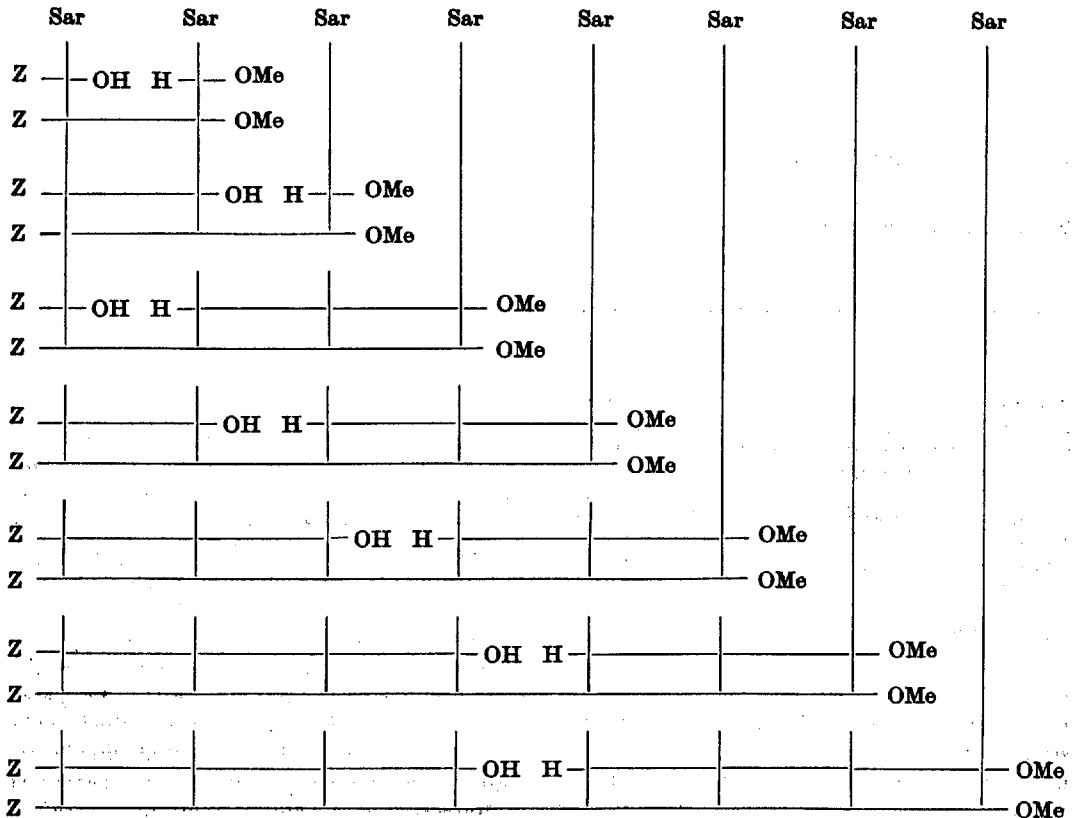
This paper describes in detail the syntheses together with additional results from the conformational investigation.

SYNTHESES

The different linear peptides were prepared by conventional methods (Scheme 1). The benzyloxycarbonyl group was used for protection of the amino function, the methyl ester for the carboxyl group and *N,N'*-dicyclohexylcarbodiimide as the coupling reagent. In the coupling reactions, which could all be carried out in dichloromethane, a tendency for acylurea products to form was observed but this could be minimised by using a low starting temperature (-15°C) and by adding the carbodiimide in portions. It is well known that simple dipeptide esters readily form diketopiperazines on liberation of the amino group and therefore it was expected that molecules, with *N*-methyl substituent, would readily show this phenomenon. Indeed attempts to remove the *Z*-group from *Z*-Sar-Sar-OMe led to cyclic dimer, even in the presence of hydrochloric acid. Consequently the tripeptide was built up by coupling to the *N*-protected dipeptide acid. This complication has also been observed for larger peptides by Meienhofer,⁵ who reported that the tripeptide *D*-valyl-*L*-prolyl-sarcosine splits off sarcosine and gives cyclo(*D*-valyl-*L*-prolyl) on storing, but was not encountered in the present study. Nevertheless, as a precaution all free amine peptides were used immediately on isolation.

For the cyclisation (Scheme 2) the methyl ester group was removed by alkaline hydrolysis and replaced by the 2,4,5-trichlorophenyl ester group by the use of dicyclohexylcarbodiimide,

* For the assignment of stereochemistry around the amide partial double bond only the atoms of the peptide chain are considered. Thus *cis* corresponds to *E* and *trans* to *Z* for all molecules considered here.

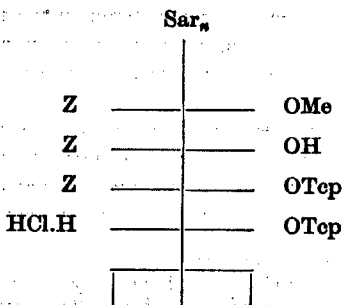


Scheme 1.

whereafter the Z-group was removed by hydrogenolysis. The resulting peptide active ester was cyclized in pyridine using a modification of Schwyzer's method,⁶ with relatively rapid addition (ca. 1 h) and subsequent refluxing for only 1 h. After passage through an ion-exchange column to remove non-cyclized

material, the cyclic products were isolated by either vacuum sublimation or crystallisation.

Each cyclisation reaction gave not only the corresponding cyclic compound but also other cyclic peptides of sarcosine with both larger and smaller rings. The yields which were relatively high, varied with size of the linear peptide but were unfortunately not particularly reproducible from experiment to experiment. Typical results are shown in Table 1. It can be seen that the cyclic dimer is the major byproduct in all cyclisations. The possibility that this arose *via* a transannular reaction in the cyclic products could be excluded since cyclic tri-, tetra-, and hexapeptides were recovered unchanged after heating at 150 °C for 3 h and also the tetrapeptide after boiling for 5 h in pyridine. Diketopiperazine formation was found to be very prominent during cyclisations of a series of heterodetic tetrapeptides.⁷



Scheme 2. n = 3, 4, 5, 6, 7, 8.

Table 1. Yields (%) of cyclic peptides formed upon cyclisation of the different linear peptides.

c-Sar _n , n =	2	3	4	5	6	7	8
From HCl.H - Sar ₂ - OTcp	20	16	1.5		10		
» HCl.H - Sar ₃ - OTcp	21		5.8				3
» HCl.H - Sar ₄ - OTcp	30		1	6.5			
» HCl.H - Sar ₅ - OTcp	31		1		4.2		
» HCl.H - Sar ₆ - OTcp	14		6			3.8	
» HCl.H - Sar ₇ - OTcp	22		0.5				5.5

The yields of the cyclic dipeptide are calculated on the basis that only one molecule of cyclic dipeptide is formed per oligomer.

In some cases only cyclic dipeptides were isolated, especially where the second amino acid in the peptide chain was *N*-methylated. The mechanism was found to be the same as that reported by Meienhofer,⁸ a nucleophilic attack by the free amino group on the second carbonyl carbon in the chain (Fig. 1). This removal of two units can obviously occur several times in the higher homologues; however, the yields in the table have been calculated on the basis that only one cyclic dipeptide is formed per oligomer. Minor quantities of cyclotetrasarcosyl were also isolated from every cyclisation. This probably originates from an analogous reaction but where the peptide chain is folded in such

a way that the amino group comes close to the fourth carbonyl carbon, and is in accord with the observation that cyclotetrasarcosyl has a very stable conformation with a *cis,trans,cis,trans* sequence of the amide groups. The tetramer isolated from the tripeptide reaction arises from the hexapeptide formed by doubling of the chain. No other cyclic peptides, such as tri- and pentapeptides, were formed by chain cleavage.

Doubling reactions are well known and are most marked for tripeptides with unsubstituted nitrogens, which normally give only cyclic hexamers. Doubling reactions were first observed with tri- and pentapeptides, and hydrogen bonding between two molecules, prior to cyclisation was suggested.⁹ However, later studies with depsipeptides^{9,10} and peptides which exclude hydrogen bonding^{10,11} also demonstrated this phenomenon and indicated a simple coupling to the linear hexa- or decapeptide before cyclisation. Conformationally it is easier for cyclisation to occur with the larger peptides. The only exceptions where cyclic tripeptides have been found are when the amino acid is *N*-substituted and the peptides more readily allow *cis*-amide bonds. Thus only cyclic tripeptides from proline¹² and from combinations of proline and hydroxyproline¹³ or sarcosine¹⁴ have been described previously. However, even here dimerisations occurred. In the present study dimerisation was found to take place with tri-, tetra-, and penta-peptides, but the yields of doubling products were low and only the dimers from the tri- and tetra-peptides were isolable. Rothe,¹⁴ on the other hand, has reported that cyclohexasarcosyl is formed in relatively high yield (38%), together

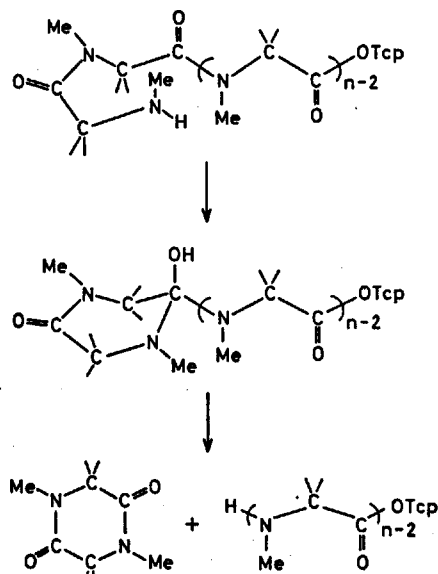


Fig. 1.

Table 2. Properties of c-Sar_n.

<i>n</i> =	2	3	4	5	6	7	8
Sublim. temp. at 0.01 mmHg, °C	100	150	200	240	270	280	280
M.p., °C	147	221	> 350	255	315	296	338
Mol ion in mass spectrum	142	213	284	355	426	497	586
C=O str.freq. IR. (cm ⁻¹) in CHCl ₃	1670	1650	1670, 1650	1675, 1640	1670, 1645 ^a	1670(1650)	1675(1650)

^a in KBr

with cyclonona- and dodecapeptide, when the tripeptide of sarcosine is cyclized using the pentachlorophenyl ester as active ester.

PROPERTIES OF THE PEPTIDES

The majority of the linear peptides remained as foamy solids. This indicates the presence of several conformers which may be due to the presence of both *cis* and *trans* amide bonds in the chain. It is possible to record the NMR spectra of the crystal conformations by dissolving the peptides at low temperature (see below), and, in fact, the crystalline tripeptide, Z-Sar-Sar-Sar-OMe, when dissolved at -70 °C, shows only one conformer containing each amide bond in a single configuration, *cis* or *trans*. On raising the temperature, the spectrum becomes more complex due to *cis-trans* isomerism of the peptide bonds. The corresponding non-crystalline heptapeptide shows a complex spectrum also when dissolved at low temperature.

The cyclic peptides (Table 2), however, are well defined solids with sharp melting points, sublimable and stable to heat, and they give the molecular ion in the mass spectrometer. They are soluble in chloroform, the cyclic di- and tripeptides also in benzene, in which solvent their dipole moments were measured. The infrared spectra show in several compounds separated carbonyl absorptions, but it was not possible to assign these unambiguously to *cis* and *trans* peptide bonds or to particular locations in the molecule.

The NMR spectra of the cyclic peptides show several common features and yielded considerable information. Different techniques were employed to aid spectral analysis and to provide information on the conformations of the in-

dividual cyclic peptides. Aromatic solvent induced shifts¹⁵ were used to resolve overlapping signals. Dropwise addition of deuterio-benzene caused most of the signals to move to higher field to differing degrees, but did not alter the coupling constants or conformations. Surprisingly, the cyclic peptides in this series possess high conformational barriers to ring inversion which is coupled with *cis,trans* configuration exchange in the larger rings, and this enables an unambiguous correlation between crystal and solution conformations. For example, a coalescence temperature of 30 °C (which is lower than for most of the cyclic peptides) will, according to the Eyring equation¹⁶ correspond to an inversion barrier of about 15 kcal mol⁻¹ and the life-time for a conformer at -70 °C will be about one hour. Whereas at the coalescence temperature the life-time of the conformer will be of the order of 10⁻³ s. Thus if the crystals are dissolved at -70 °C the spectrum can be recorded before any conformational change can take place. Furthermore, warming the sample and recording the spectrum will reveal such changes if any. This technique has previously been applied to simple amide systems.¹⁷⁻¹⁸

Since the NMR spectra could, due to the high barrier to ring inversion, be recorded below the coalescence temperature of the rings, analysis of the methylene quartets shows that the geminal coupling constants fall into two main categories, one with *J* of 14 Hz and the other with *J* of 18 Hz, the pairs of signals with the smaller coupling constants being usually more widely separated. The geminal coupling constant for a methylene group adjacent to a carbonyl is reported to depend on its relative orientation to the carbonyl group,¹⁹ the largest value being found when the dihedral angle

of $R-C_{CO}-C_{\alpha}-R'$ is 0 or 180° and the smallest for 60 or 120° . The observed values in the sarcosine series indicates that the skeletal dihedral angles of $N-C_{CO}-C_{\alpha}-N$ display only a limited number of values and that these are repeated in all rings. This is confirmed by the crystal structure of cyclotetra-,²⁰ cyclopenta-,^{4,21} cyclohepta-,^{21a} and cyclooctasarcosyl^{2,22} which have been determined by P. Groth, and which show that the $N-C_{CO}-C_{\alpha}-N$ dihedral angles follow an *anti-gauche* pattern.

DISCUSSION OF INDIVIDUAL CYCLIC PEPTIDES

Cyclodisarcosyl (Fig. 2) shows a simple NMR spectrum with one line for the *N*-methyl and

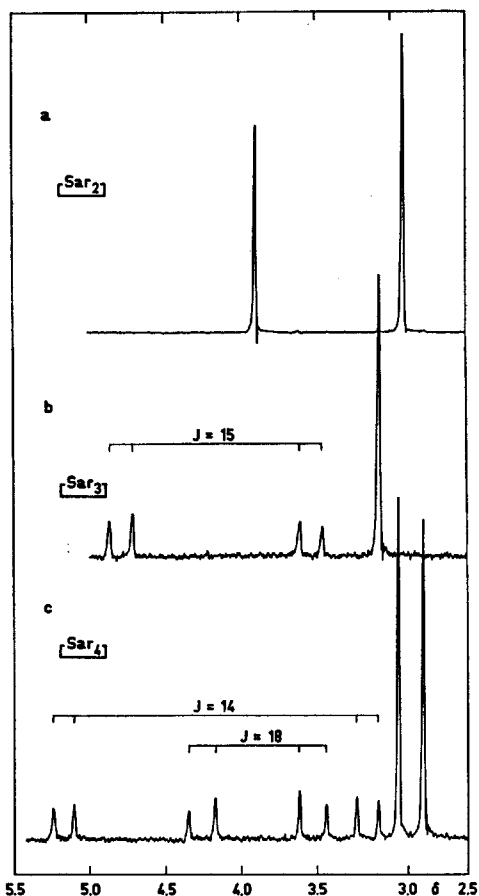


Fig. 2. The 100 MHz NMR spectrum of a, cyclodisarcosyl, b, cyclotrisarcosyl, and c, cyclotetrasarcosyl in $CDCl_3$ at $35^\circ C$.

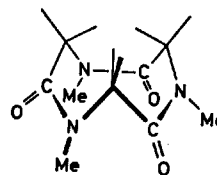


Fig. 3. The conformation of cyclotrisarcosyl.

one for the methylene protons. No broadening was observed down to $-70^\circ C$, and thus the ring is either inverting rapidly or is close to planar. The small dipole moment (1.13 D in benzene) suggests a boat type conformation in solution. The X-ray crystallographic analysis²³ revealed a flattened chair with a $CC_{\alpha}NC$ dihedral angle of 7° and the *N*-methyl carbons in a common plane with the amide groups.

Cyclotrisarcosyl (Fig. 2) shows one *N*-methyl signal and one methylene quartet, intensity 9:6, and the quartet coalesces to a single line at $135^\circ C$ (60 MHz, 1,2-dichlorobenzene solvent). This clearly shows that only one conformer is present in solution and that this contains only one type of amide bond. Since all in *trans* is excluded for a nine-membered ring, all must be *cis*. The coupling constant for the methylene protons is 15 Hz and is in accord with a *gauche* type dihedral angle for $C_{CO}-C_{\alpha}$, and the high dipole moment (4.66 D in benzene) conforms with the three carbonyl groups being on the same side of the ring. The crown conformation (Fig. 3) is the only one consistent with these data. The barrier to ring inversion,²⁴ (20.1 kcal mol⁻¹) is in this case accidentally in the same region as for *cis-trans* isomerism of amides.

Cyclotetrasarcosyl (Fig. 2) shows two *N*-methyl singlets, intensity 6:6, and two methylene quartets, intensity 4:4, and therefore exists in one conformation in solution. By symmetry arguments a *cis, trans, cis, trans* configuration of the amide bonds in a centrosymmetric molecule was suggested.³ The crystal structure²⁰ (Fig. 4) is virtually identical to that proposed, the dihedral angles of $N-C_{CO}-C_{\alpha}-N$ are 66 and 171° and correspond to the observed geminal coupling constants of 14 and 18 Hz. The NMR lines of this molecule coalesce only at a surprisingly high temperature, the CH_2 -quartets start to broaden at $155^\circ C$ (60 MHz, quinoline solvent) and at

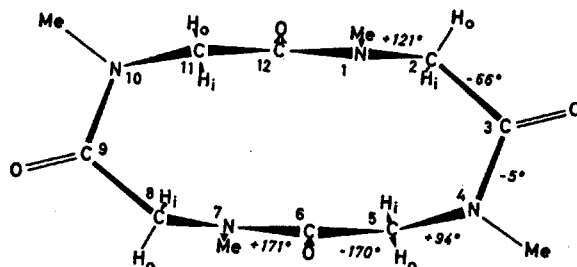


Fig. 4. The crystal conformation of cyclotetrasarcosyl.

about 200 °C the spectrum consists of a single line for the CH_2 's and a single line for the *N*-methyl protons. In our preliminary communication² we suggested that this high resistance to ring inversion might be due to transannular interactions, such transannular interactions having been reported between the ester oxygen and ketone carbonyl in the crystal of the 10-membered-ring lactone of 9-hydroxy-6-oxononanoic acid²⁵ and between amino and keto-groups in some medium ring compounds.²⁶ However, the X-ray structure of cyclotetrasarcosyl²⁰ shows that the distance across the ring from carbonyl carbon to nitrogen is too large (3.08 Å) for such an interaction. Moreover, the analogous cyclic tetrapeptide, Sar-Gly-Sar-Gly, which adopts the same conformation as cyclotetrasarcosyl both in solution²⁷ and in the crystal²⁸ with the two *NH*-amide groups placed in the *trans* positions, has approximately the same transannular distance (3.00 Å). A longer distance was expected on replacing *N*- CH_3 with *NH* due to the lower basicity of the *N*-H. The barrier to ring inversion is now much lower (coalescence at *ca.* 20 °C). This indicates that the high barrier to ring inversion of cyclotetrasarcosyl is due more to the steric influence of the *N*-methyl groups.

Recently a naturally occurring cyclic tetrapeptide, tentoxin, cyclo(*N*-methyl-dehydrophenylalanyl-L-leucyl-glycyl-*N*-methyl-L-alanyl) has been found to take up an identical conformation with its two *N*-methylated amide groups in the *cis* positions.^{29,29a}

Cyclooctasarcosyl (taken out of sequence since the conclusions form the basis for the discussion of cyclopenta-, cyclohexa-, and cycloheptasarcosyl) shows only one conformer in solution; four equally intense *N*-methyl lines

and four CH_2 -quartets (*J* 16 to 18 Hz) become resolved in the spectrum in chloroform (Fig. 5) on gradual addition of deuteriobenzene. The ring shows an unexpectedly high barrier to ring inversion, the CH_2 -quartets begin to broaden at *ca.* 40 °C (60 MHz, chloroform solvent) and result in a sharp line together with a sharp line from the *N*-methyl protons at 120 °C, sealed tube. The solution conformation is shown to be the same as that in the solid by NMR and IR methods. Thus dissolution of the crystals in CHFCl_2 at -80 °C gives an NMR spectrum which does not change on warming to room temperature. Also the IR spectrum in the transparent regions of a chloroform solution is identical to that of the solid in KBr. The X-ray determination (Fig. 6) shows an open ring conformation^{3,22} with a cluster of four water molecules, although the

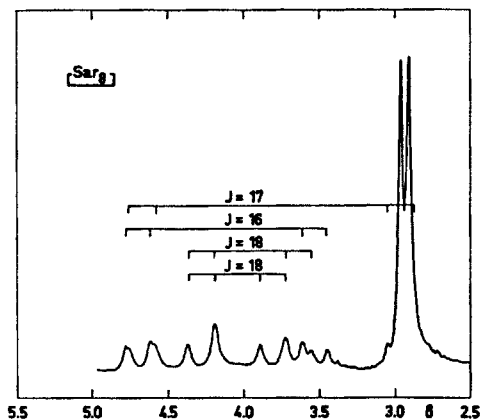


Fig. 5. The 100 MHz NMR spectrum of cyclooctasarcosyl in CDCl_3 (contg. a trace of TFA) at 35 °C. The splitting pattern was deduced by hexadeuteriobenzene addition and spin-decoupling.

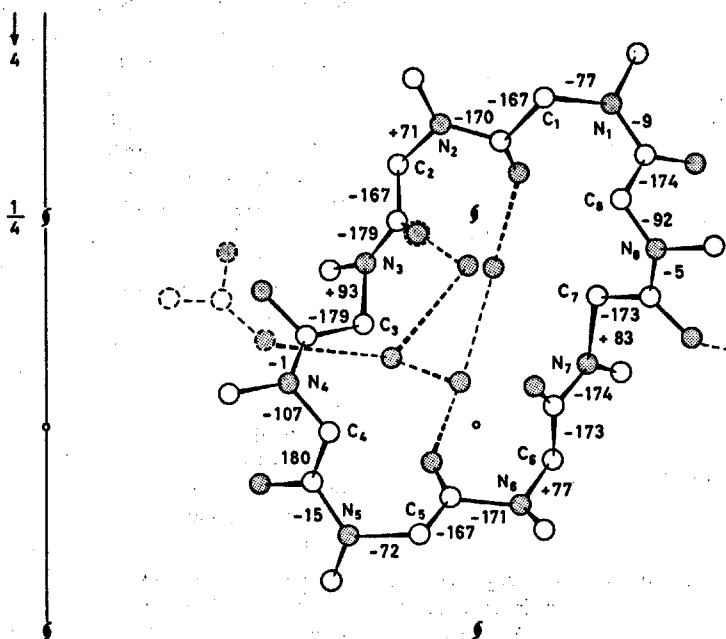


Fig. 6. The crystal structure of cyclooctasarcosyl. Open circles are carbon atoms, filled circles nitrogen (marked) or oxygen atoms. Dihedral angles refer to the ring skeleton.

crystals were grown from methanol. The molecule possesses approximate two-fold symmetry with the configuration sequence *cis,cis-trans,trans,cis,cis,trans,trans* of the amide groups and with a deviation from planarity of up to 15° . The dihedral angles in the $N-C_{CO}-C_\alpha-N$ bonds are close to *anti* (between 167 and 180°) and correspond well with the large observed coupling constants, the $C_{CO}-C_\alpha-N-C_{CO}$ dihedral angles are relatively constants ($71-107^\circ$), and moreover the peptide chain is folded in helical segments. The two *trans* portions [C(1) to N(4) and C(5) to N(8)] form a right-handed threefold screw axis with a residue repeat distance of 3.1 \AA , which corresponds well to the left-handed helix found in poly-L-proline II,²⁰ where the amide bonds are *trans* and the translation 3.12 \AA ; a similar situation is found for polyglycine II.²¹ The two *cis* portions [C(3) to N(6) and C(1) to N(2)] also form part of a right-handed helix with a threefold screw axis but with a shorter residue repeat distance, 2.3 \AA ; this resembles the helix found in poly-L-proline I²² where the amide bonds are *cis* and the translation 1.9 \AA .

This specific folding of the chain may explain

the relatively high resistance to ring inversion. Transannular interactions as originally suggested were discounted when the X-ray analysis showed the transannular distance to be too great for such an interaction.

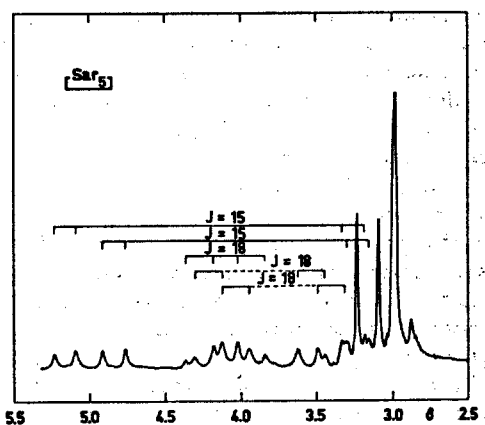


Fig. 7. The 100 MHz NMR spectrum of cyclopentasarcosyl in $CDCl_3$ at $35^\circ C$. The splitting pattern was partially deduced from spin-decoupling and the two quartets shown by dotted lines are presumed to be as shown from the hexadeuteriobenzene addition.

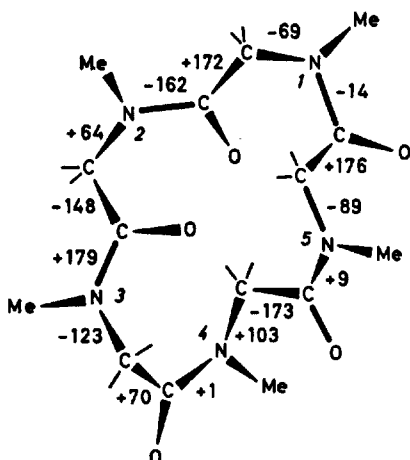


Fig. 8. The crystal conformation of cyclopentasarcosyl.

Cyclopentasarcosyl shows on dropwise addition of benzene to the chloroform solution (Fig. 7) five equally intense *N*-methyl lines and five methylene quartets, three with large coupling constant (18 Hz) and two with smaller coupling constants (14 and 15 Hz). Some weak additional lines (less than 10%) are visible in the *N*-methyl region and may be assigned to a second conformer. Dissolution of the crystals in deuteriochloroform at -50°C gives an NMR spectrum which shows only the lines corresponding to the dominant conformation in the room temperature spectrum. The additional signals begin to appear at about -30°C and this confirms that these are due to a second solution conformation. The coalescence temperature is relatively high, the CH_2 -quartets start to broaden at 40°C (60 MHz, chloroform solvent) and at 130°C the spectrum consists of a single line for the CH_2 's and a single line for the *N*-methyl protons. The crystal structure²¹ (Fig. 8) was determined on crystals grown from methanol but which contained two molecules of water. The *cis-trans* pattern of the amide groups was quite unexpected, with a succession of three *cis* and two *trans* where two of the *cis* and one *trans* deviate significantly from planarity, 9, 14, and 18° , respectively. Three of the dihedral angles in the $\text{C}_{\text{CO}}-\text{C}_{\alpha}$ bonds are close to *anti* and may be assigned to the three large coupling constants, and the two other 70 and 148° to the smaller ones. The dihedral

angles in the $\text{C}_{\alpha}-\text{N}$ bonds vary between 64 and 123° . Again the chain is folded in helical segments and resembles those found in cyclo-octasarcosyl. If the pentapeptide ring is cut open in the $\text{C}_{\text{CO}}-\text{C}_{\alpha}$ bond of residue 3 and the ends spread somewhat apart, the resulting chain 3,2,1,5,4 is directly superimposable on the corresponding part of the octapeptide ring skeleton (residues 3,2,1,8,7).

Cyclohexasarcosyl is less soluble in organic solvents than the others in this series. A sample for NMR can be dissolved in chloroform containing a trace of trifluoroacetic acid. The spectrum is complex with broad methylene and *N*-methyl lines and these do not become any simpler on cooling to -70°C . On addition of benzene more than six *N*-methyl lines appear indicating that more than one conformer is present in solution. The spectrum of the crystal conformation (Fig. 9), is obtained by dissolving a sample in CHFCl_2 plus a trace of TFA at -80°C , and shows three equally intense *N*-methyl signals and three CH_2 -quartets with coupling constants of 18, 18, and 15 Hz, and no new lines being resolved on dropwise addition of benzene (added as a 1:1 mixture of $\text{C}_6\text{D}_6/\text{CHFCl}_2$ at -60°C). This is consistent with either centro- or twofold-symmetry in the molecule, and the coupling constants show four of the $\text{N}-\text{C}_{\text{CO}}-\text{C}_{\alpha}-\text{N}$ dihedral angles to be *anti* and the other two to be smaller, which can

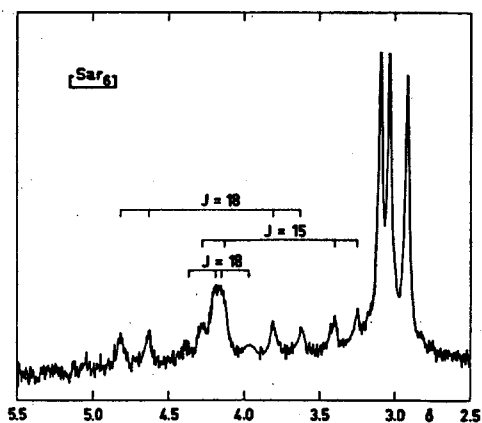


Fig. 9. The 100 MHz NMR spectrum of cyclohexasarcosyl in CHFCl_2 (contg. a trace of TFA) at -60°C (dissolved at this temp.). The splitting pattern was deduced by spin-decoupling.

be accounted for by specific folding of the chain into helical segments as found for cycloocta- and cycloheptasarcosyl with either *cis,cis-trans,cis,cis,trans* or *trans,trans,cis,trans,trans,cis* arrangement of the peptide units. On warming to about -30°C the methylene region becomes unsharp and new *N*-methyl signals appear due to *cis-trans* interchange and development of new conformers. The coalescence of $\text{N}-\text{CH}_3$ and CH_2 take place between 50 and 100°C and leaves one pair of singlets.

Cycloheptasarcosyl is easily soluble in CDCl_3 and has an NMR spectrum with broad methylene signals which do not sharpen upon cooling to -70°C . Benzene addition resolves seven main *N*-methyl signals together with several less intense signals which indicates that one major conformer is present, together with one or more minor conformers. The NMR spectrum of the crystal conformation (Fig. 10) which is obtained in the usual way by dissolution in CHFCl_2 at -80°C shows three *N*-methyl lines, intensity 9:9:3 and seven partially resolved quartets of which one has a small coupling constant (14 Hz) and the other six seem to have large coupling constants (18 Hz). No appreciable resolution of the spectrum was achieved by dropwise addition of benzene at -60°C (added as a 1:1 mixture of $\text{C}_6\text{D}_6/\text{CHFCl}_2$). At about -30°C new *N*-methyl lines appear and the methylene quartets begin to broaden, but two sharp lines are not obtained until 110°C

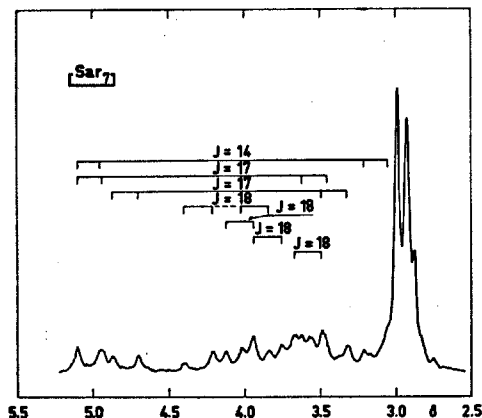


Fig. 10. The 100 MHz NMR spectrum of cycloheptasarcosyl in CHFCl_2 at -80°C (dissolved at this temp.). Only limited spin-decoupling was feasible and therefore a complete interaction of signals not possible.

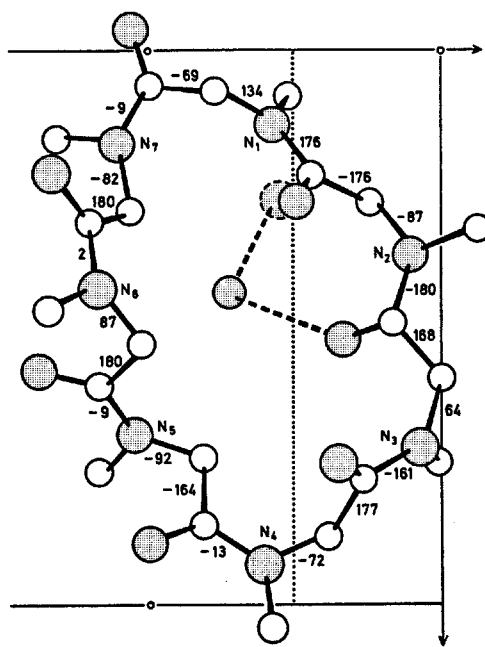


Fig. 11. The crystal structure of cycloheptasarcosyl. Open circles are carbon atoms, filled circles nitrogen (marked) or oxygen atoms. Dihedral angles refer to the ring skeleton.

(60 MHz, chloroform solution, sealed tube).

The crystal structure^{11b} (Fig. 11) was determined on crystals grown from methanol but which contained one molecule of water. The *cis-trans* pattern of the amide groups was again somewhat unexpected with a succession of four *cis* and three *trans* where the largest deviation from planarity (19°) is found in a *trans* amide bond. Six of the dihedral angles in the $\text{C}_{\text{CO}}-\text{C}_\alpha$ bonds are close to *anti* (between 164 and 180°) and the seventh close to *gauche* (69°). The dihedral angles in the $\text{C}_\alpha-\text{N}$ bonds lie between 64 and 134° with the majority close to 90° . Again it appears as though the chain is folded into helical segments such that the four *cis* units form two independent similar helical fragments and two of the *trans* linked units form a third.

COMPLEXATION

Since it is well known that many cyclic compounds form complexes with alkali metal salts^{33,34} some experiments were undertaken with cyclohexa-, cyclohepta-, and cyclo-

octasarcosyl to assess their complexing ability. Addition of hexamer to KNCS in methanol gave a precipitate which was insoluble in organic solvents. The NMR spectrum in D₂O was identical to that of pure hexamer, but here the complex may be expected to be largely dissociated. With the heptamer no tendency for complex formation was observed; for instance, KNCS did not dissolve in a chloroform-solution of cycloheptasarcosyl and no precipitate formed from acetone. The octamer, however, dissolved in acetone on addition of KNCS and shows after evaporation an NMR spectrum (in CD₃OD) which differs significantly from that of the free octamer, now with broad methylene lines which do not sharpen even at -60 °C. The barrier to ring inversion is also lower as the CH₂-quartets show one broad line at 30 °C (*cf.* uncomplexed at 100 °C). Apparently, the favoured conformation of cyclooctasarcosyl has been altered and now probably at least six of the carbonyl groups are orientated towards the metal atom as is found in the potassium complex of enniatin B²⁵ and of valinomycin.²⁶ The cyclic peptides were readily recovered by passing the complex through a mixed bed (strong acid and strong base) ion-exchange resin.

CONCLUSION

The high conformational homogeneity and high barriers to ring inversion found for the cyclic oligomers of sarcosine are at first sight surprising since the absence of α -substituents and hydrogen bonding, and the possibility for both *cis* and *trans* amide bonds, would be expected to result in a large number of competing conformations. This study has, however, revealed a number of striking similarities between the various rings and suggests that other forces are significant in defining conformations. In the small rings steric effects due to the *N*-methyl groups seem to be dominant, but in the larger rings there appears to be a considerable tendency for residues to form helical segments. This latter effect is most clearly demonstrated in the cyclic octamer where the formation of the potassium complex lowers the barrier to ring inversion; it is virtually certain that the complexation alters the favoured conformation. It is also apparent

that the three different types of bonds present in the ring skeleton (C_{CO}-N, C_{CO}-C _{α} , and C _{α} -N) take up only a limited number of orientations relative to one another, but at present it is not certain whether this is due solely to the helical influence or to some other factor. The observed dihedral angles for N-C_{CO}-C _{α} -N are either close to *anti* (180°) or *gauche* (60°), the only observed exception being that cyclopentasarcosyl has one intermediate value (148°); this is required to allow the remaining residue to form helices. The C _{α} -N bonds show a wider variation of dihedral angle but are, in the main, grouped around 90°. The amide bonds exist in both *cis*- and *trans*-forms, except in the smallest cyclo di- and trisarcosyl which are all *cis*. The maximum deviation from planarity found for the *cis*-amide group is 15° and for *trans* 19°. It is also noteworthy that all the carbonyl groups of the *cis* amide linkages are directed out of the ring whilst those from the *trans* are directed into the ring.

EXPERIMENTAL

Sarcosine was obtained from Merck Chemical Company. Solvents used in the reactions were of analytical grade, *N,N*-dimethylformamide and pyridine were dried over CaH₂ and distilled, the light petroleum had b.p. 40-60 °C. General methods of preparation are described where appropriate. Evaporations were performed in a rotary evaporator.

Several of the linear peptide derivatives were either viscous oils or noncrystalline 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, ethylacetate-chloroform 1:2 (A), 10% methanol-chloroform (B), 15% methanol-chloroform (C), 20% methanol-chloroform (D), methanol:chloroform:water 70:20:10 (E), ethanol:water:acetic acid 80:10:10 (F), detection in iodine vapor.

The cyclic peptides were separated from the linear ones by passage through a monobed ion-exchange column (Amberlite, MB-1, analytical grade), using methanol, or a mixture of methanol and water (1:1), as eluent.

Samples for elemental analysis of the methyl esters, Z-Sar_n-OMe, were purified on a silica gel column, eluted with chloroform followed by chloroform added increasing amount of methanol 2-10%.

Purification of the acids, Z-Sar_n-OH, was attempted by extraction from an organic layer (dichloromethane or chloroform) into an alkaline

aqueous layer, acidification and re-extraction into an organic layer, followed by drying over MgSO_4 and evaporation, but with limited success. The dicyclohexylammonium salts were also prepared but in most cases without satisfactory results.

With the trichlorophenyl esters, $Z\text{-Sar}_n\text{-OTcp}$, a satisfactory purification on silica column could not be achieved due to transesterification with the methanol in the eluting solvent. Some of these esters contained dicyclohexylurea which was most readily removed after cyclisation.

Cyclotrisarcosyl

Benzylloxycarbonylsarcosylsarcosine methyl ester (Z-Sar₂-OMe). Sarcosine methyl ester hydrochloride²⁷ (28 g = 200 mmol) (prepared from sarcosine in methanol containing thionyl chloride) was suspended in dichloromethane (500 ml), triethylamine (20.5 g = 200 mmol) added and the precipitated triethylamine hydrochloride filtered off. Benzylloxycarbonylsarcosine²⁸ (44.5 g = 200 mmol) in dichloromethane (400 ml) was combined with the filtrate, stirred and cooled to -15°C and dicyclohexylcarbodiimide (43.5 g = 210 mmol) added in portions over 30 min. The temperature was raised slowly to 0°C and the reaction mixture kept in an icebath which was allowed to attain room temperature during 15 h. The precipitated dicyclohexylurea was filtered off, the filtrate diluted with dichloromethane (500 ml) and washed successively with 0.1 N HCl, water, 0.5 M NaHCO_3 , water and finally dried over MgSO_4 and evaporated. The residual oil was dissolved in ether and precipitated with light petroleum to leave a viscous oil (58 g = 94%). Homogeneous by TLC, R_F 0.3 (A), R_F 0.8 (B). 0.3 g was further purified on a silica gel column. (Found: C 58.31; H 6.49; N 8.71. $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_5$ requires C 58.43; H 6.54; N 9.09).

Benzylloxycarbonylsarcosylsarcosine (Z-Sar₂-OH). To an ice-cooled, stirred solution of benzylloxycarbonylsarcosylsarcosine methyl ester (50 g = 160 mmol) dissolved in a mixture of methanol (20 ml) and water (60 ml) was added 1 N sodium hydroxide (160 ml) in portions, and the solution kept at room-temperature (3 h) until the reaction was complete (TLC). The mixture was concentrated at 30°C to ca. 40 ml, acidified with 2 N hydrochloric acid to pH 2 and extracted several times with dichloromethane. The organic layer was washed with a small amount of water, dried and evaporated to give an oil which was treated with ether and light petroleum. The resulted viscous oil (42 g = 88%) was homogeneous by TLC, R_F 0.8 (F). (Found: C 56.74; H 6.30. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$ requires C 57.13; H 6.17).

The dicyclohexylammonium salt was pre-

pared by dissolving 0.3 g acid in dichloromethane together with excess of dicyclohexylamine and evaporated. The residual oil crystallized from a mixture of acetone-ether-light petroleum to give a white solid m.p. $130\text{--}132^\circ\text{C}$. (Found: C 65.64; H 8.41; N 8.87. $\text{C}_{22}\text{H}_{40}\text{N}_2\text{O}_5$ requires C 65.80; H 8.49, N 8.85).

Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (Z-Sar₃-OMe). Sarcosine methyl ester hydrochloride (16 g = 115 mmol) was suspended in dichloromethane (500 ml) and triethylamine (11.6 g = 115 mmol) added. The filtered solution was mixed with benzylloxycarbonylsarcosylsarcosine (33.8 g = 115 mmol), cooled to -15°C and dicyclohexylcarbodiimide (25 g = 121 mmol) added in portions with stirring. The reaction conditions and way of isolating the protected peptide were as described for $Z\text{-Sar}_2\text{-OMe}$. The residual oil (36 g) crystallized from methanol-ether and was recrystallized from the same solvents (33 g = 75%). M.p. $101\text{--}103^\circ$, TLC, R_F 0.55 (B). (Found: C 56.82; H 6.85; N 10.78. $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_6$ requires C 56.98; H 6.64; N 11.08).

Benzylloxycarbonyl(sarcosyl)₂sarcosine (Z-Sar₃-OH). To an ice-cooled solution of benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (14 g = 37 mmol) in methanol (20 ml) and water (60 ml) was added 1 N sodium hydroxide (38 ml) and kept at room temperature for 2.5 h. Worked up as described for $Z\text{-Sar}_2\text{-OH}$ to give a noncrystalline solid (11.5 g, 85%) which softened at about 45°C TLC, R_F 0.75 (F).

Benzylloxycarbonyl(sarcosyl)₂sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₃-OTcp). To an ice-cooled and stirred solution of benzylloxycarbonyl(sarcosyl)₂sarcosine (2.9 g = 8 mmol) and 2,4,5-trichlorophenol (2 g = 10 mmol) in dichloromethane (50 ml) was added dicyclohexylcarbodiimide (1.8 g = 8.7 mmol) and the mixture was allowed to attain room temperature during 15 h. The precipitated dicyclohexylurea was filtered off, the filtrate evaporated and the residue treated twice with ether-light petroleum, which was decanted off, and the residue evaporated to dryness to give a white solid (4 g = 92%), which had an NMR consistent with the structure, TLC, R_F 0.6 (B).

A sample (0.2 g) was purified on a silica gel column, eluted with chloroform followed by chloroform added 2% methanol. (Found: C 51.00; H 4.47. $\text{C}_{13}\text{H}_{24}\text{N}_2\text{O}_6\text{Cl}_3$ requires C 50.71; H 4.44).

Cyclisation. A solution of benzylloxycarbonyl(sarcosyl)₂sarcosine 2,4,5-trichlorophenyl ester (2 g = 3.7 mmol) in methanol (100 ml) and concentrated hydrochloric acid (0.2 ml) was purged with nitrogen gas and 5% palladium on charcoal (0.4 g) added. Hydrogen gas was passed through the mixture until evolution of CO_2 ceased (30 min) [tested with $\text{Ba}(\text{OH})_2$]. The solution was filtered through Celite and evaporated at ca. 30°C to yield a white solid (1.45 g = 88%).

Part of the resulting (sarcosyl)₂sarcosine 2,4,5-trichlorophenyl ester hydrochloride (0.65 g=1.46 mmol) in dimethylformamide (50 ml) was added dropwise under vigorous stirring to pyridine (500 ml) at 115°C over a period of 2 h and stirred for another 1 h. After evaporation the residue was dissolved in methanol-water (1:1) (50 ml), passed through an ion-exchange column and eluted with methanol-water (only methanol can also be used). The first eluate (200 ml), which contained most of the cyclic peptides, was evaporated and the residue taken into methanol (5 ml). Undissolved material was filtered off (3 mg) which appeared to be pure cyclotetrasarcosyl. The filtrate was evaporated, acetone (5 ml) added and kept in a refrigerator overnight. The precipitate which now mostly contained cyclodisarcosyl together with some cyclohexasarcosyl was filtered off, the residue after evaporation washed with ether and sublimed in a glasstube at reduced pressure and gradually raising the temperature.³⁹ The different precipitations were then sublimed separately. Yields of isolated cyclic peptides: Cyclotrisarcosyl (16 %) sublim.temp. 150°C/0.01 mmHg, m.p. 221°C, TLC, R_F 0.5 (C), m/e 213. (Found: C 50.61; H 6.94; N 19.84. C₉H₁₆N₃O₃ requires C 50.69; H 7.09; N 19.71) and in addition cyclodisarcosyl (20 %), cyclotetrasarcosyl (1.5 %) and cyclohexasarcosyl (9.3 %).

Cyclotetrasarcosyl

Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (Z-Sar₄-OMe). Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (20 g=528 mmol) in methanol (500 ml) was purged with nitrogen gas and 5 % palladium on charcoal (2 g) added. Hydrogen was bubbled through the mixture (3 h), the product filtered through Celite and evaporated. The residual oil, (sarcosyl)₂sarcosine methyl ester (12 g=49 mmol=93 %) was examined on NMR and TLC and without storing dissolved together with benzylloxycarbonylsarcosine (11 g=49 mmol) in dichloromethane (200 ml), cooled to -15°C and dicyclohexylcarbodiimide (12 g=58 mmol) added. The reaction conditions and way of isolation were as described for Z-Sar₃-OMe, and resulted in a viscous foam (19 g=86 %) of the protected tetrapeptide. The NMR was consistent with the structure, and homogeneous by TLC, R_F 0.35 (B), R_F 0.75 (C).

Benzylloxycarbonyl(sarcosyl)₂sarcosine (Z-Sar₄-OH). Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (11 g=25.2 mmol) was hydrolysed in methanol (20 ml) and water (40 ml) with 1 N sodium hydroxide (26 ml) for 2.5 h in the same way as described for Z-Sar₃-OH. After evaporation and acidification the aqueous layer was extracted with chloroform and worked up to give a white foam (9.5 g=87 %) which displayed ap-

propriate NMR spectrum and TLC, R_F 0.65 (F).

Benzylloxycarbonyl(sarcosyl)₂sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₄-OTcp). Benzylloxycarbonyl(sarcosyl)₂sarcosine (2.2 g=5 mmol), 2,4,5-trichlorophenol (1.5 g=7.6 mmol) and dicyclohexylcarbodiimide (1.2 g=5.8 mmol) in dichloromethane (50 ml) were reacted together for 15 h as for Z-Sar₂-OTcp. The work up gave a white non-crystalline solid (2.9=94 %), one main spot on TLC, R_F 0.45 (B), R_F 0.8 (D) with traces of DC-urea.

Cyclisation. Benzylloxycarbonyl(sarcosyl)₂sarcosine 2,4,5-trichlorophenyl ester (1.9 g=3.1 mmol) in methanol (200 ml) and hydrochloric acid (0.15 ml) was hydrogenated over 5 % palladium on charcoal (0.3 g) for 1 h and worked up to yield a white solid (1.4 g=2.7 mmol=88 %) of (sarcosyl)₂sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamide (50 ml) and added dropwise to stirred pyridine (700 ml) at 115°C over a period of 1 h, and stirred for another 1 h. After evaporation the residue was taken into methanol (10 ml) and undissolved material filtered off (380 mg), this appeared to be pure cyclotetrasarcosyl. The filtrate was diluted with methanol (100 ml), passed through an ion-exchange column and eluted with methanol. The first eluate (250 ml) contained most of the formed cyclic peptides while the next eluate (400 ml) also contained some dicyclohexylurea (20 mg). This was easily removed by sublimation (150°C/0.01 mmHg) when the different cyclic peptides were isolated. Yields of cyclic compounds: Cyclotetrasarcosyl (58 %), sublim. temp. 200°C/0.01 mmHg, m.p. > 350°C, TLC, R_F 0.6 (C), m/e 284. (Found: C 50.76; H 6.96; N 19.94. C₁₂H₂₀N₄O₄ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (21 % calc. from one cyclisation per chain) and cyclooctasarcosyl (3 %).

Cyclopentasarcosyl

Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (Z-Sar₅-OMe). Benzylloxycarbonylsarcosylsarcosine (6.5 g=22.1 mmol), (sarcosyl)₂sarcosine methyl ester (5.5 g=22.4 mmol) and dicyclohexylcarbodiimide (4.9 g=23.8 mmol) in dichloromethane (50 ml) were reacted together and worked up as described earlier. The isolated foam (9.8 g=86 %) displayed appropriate NMR spectrum and one spot on TLC, R_F 0.35 (B) R_F 0.6 (D). An analytical sample (0.3 g) was purified on a silica gel column. (Found: C 54.78; H 6.98; N 14.07. C₂₄H₃₆N₅O₅ requires C 55.26; H 6.76; N 13.43).

Benzylloxycarbonyl(sarcosyl)₂sarcosine (Z-Sar₅-OH). Benzylloxycarbonyl(sarcosyl)₂sarcosine methyl ester (7.8 g=14.9 mmol) in methanol (10 ml) and water (30 ml) was hydrolysed with 1 N sodium hydroxide (16 ml) in the usual way and worked up by concentrating to ca 20 ml, acidifying with 2 N

hydrochloric acid and extracting with chloroform. The acidic aqueous layer was then evaporated nearly to dryness and extracted again with chloroform, dried and evaporated. The two portions gave a solid (6.8 g = 90 %) which softened at ca. 75 °C, TLC R_F 0.55 (F).

Benzyloxycarbonyl(sarcosyl)₄sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₅-OTcp). Benzyloxycarbonyl(sarcosyl)₄sarcosine (2 g = 4 mmol), 2,4,5-trichlorophenol (1 g = 5 mmol) and dicyclohexylcarbodiimide (0.9 g = 4.4 mmol) in dichloromethane (30 ml) were reacted in the usual way and the work up resulted in a white foam (2.3 g = 86 %), one main spot on TLC, R_F 0.7 (D) with traces of DC-urea. Purification on a silica gel column afforded the analysis. (Found: C 51.15; H 5.24, C₂₂H₃₄N₅O₅Cl₃ requires C 50.70; H 4.99).

Cyclisation. Benzyloxycarbonyl(sarcosyl)₄sarcosine 2,4,5-trichlorophenyl ester (1.3 g = 1.9 mmol) in methanol (100 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.3 g) for 1 h and worked up to give a solid (1 g = 1.7 mmol = 90 %) of the (sarcosyl)₄sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamide and added dropwise to stirred pyridine (600 ml) at 115 °C over a period of 1 h and stirred for 1.5 h more. After evaporation the residue was dissolved in methanol (70 ml) and ion-exchanged. The first eluate (200 ml) was evaporated, the residue taken into acetone (10 ml) and undissolved material filtered off (200 mg) which on TLC and by sublimation was found to be almost pure cyclopentasarcosyl with traces of cyclotetrasarcosyl. The next eluate (600 ml) contained some DC-urea together with the residue purified on a silica gel column eluted with chloroform followed by addition of methanol 2–10 %. The cyclic peptides may also be isolated by sublimation.

Yields of cyclic compounds: Cyclopentasarcosyl (65 %), sublim. temp. 240 °C/0.01 mmHg, m.p. 255 °C, TLC, R_F 0.4 (C), *m/e* 355. (Found: C 50.66; H 7.21; N 19.57. C₁₅H₂₅N₅O₅ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (30 %, calc. from one cyclisation per chain) and cyclotetrasarcosyl (1 %), some cyclic decapeptide was formed, but could not be isolated by these methods.

Cyclohexasarcosyl

Benzyloxycarbonyl(sarcosyl)₅sarcosine methyl ester (Z-Sar₆-OMe). Benzyloxycarbonyl(sarcosyl)₅sarcosine (6 g = 16.4 mmol), (sarcosyl)₂sarcosine methyl ester (4.1 g = 16.7 mmol) and dicyclohexylcarbodiimide (3.7 g = 18 mmol) in dichloromethane (120 ml) were reacted together and worked up as described earlier. This gave a white powder (8.5 g = 87 %) m.p. 77 °C homogeneous by TLC R_F 0.65 (D). An analytical

sample (0.3 g) was purified on a small silica gel column. (Found: C 54.75; H 7.13; N 14.01. C₂₇H₄₀N₆O₆ requires C 54.72; H 6.81; N 14.18).

Benzyloxycarbonyl(sarcosyl)₅sarcosine (Z-Sar₆-OH). Benzyloxycarbonyl(sarcosyl)₅sarcosine methyl ester (4 g = 6.7 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (7 ml) for 2 h and worked up as described for Z-Sar₅-OH to yield a white amorphous powder (3.4 g = 87 %), softened at 98 °C, TLC, R_F 0.4 (F).

Benzyloxycarbonyl(sarcosyl)₅sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₆-OTcp). Benzyloxycarbonyl(sarcosyl)₅sarcosine (2.7 g = 3.6 mmol), 2,4,5-trichlorophenol (1.1 g = 5.6 mmol) and dicyclohexylcarbodiimide (0.85 g = 4.1 mmol) in dichloromethane (60 ml) were reacted together and worked up as described earlier. The resulting white powder (3 g = 83 %) showed one main spot on TLC, R_F 0.8 (D) with traces of DC-urea.

Cyclisation. Benzyloxycarbonyl(sarcosyl)₅sarcosine 2,4,5-trichlorophenyl ester (1.7 g = 2.6 mmol) in methanol (120 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.4 g) for 50 min and worked up to give a white solid (1.2 g = 81 %) of the (sarcosyl)₅sarcosine trichlorophenyl ester hydrochloride. Part of it (0.6 g = 0.91 mmol) was dissolved in dimethylformamide (50 ml) and added dropwise to stirred pyridine (500 ml) at 115 °C over a period of ¼ h and stirred for another 1 h. The residue after evaporation was dissolved in methanol (100 ml), ion-exchanged and the residue after evaporation taken into acetone (10 ml), filtered (130 mg) and the filtrate kept in a refrigerator overnight and filtered (10 mg). The precipitates contained mainly cyclohexasarcosyl with traces of cyclotetrasarcosyl. The solution was evaporated and the solid treated with ether and sublimed until the temperature was raised to 230 °C. The cyclic hexapeptide sublimates at 270 °C, but at this temperature the residue tends to decompose. It is therefore better to dissolve the residue in acetone, precipitate with ether and to crystallize the remaining cyclohexasarcosyl from methanol. Yields of cyclic compounds: Cyclohexasarcosyl (42 %), sublim. temp. 270 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.4 (D), *m/e* 426. (Found: C 50.67; H 7.45; N 19.80. C₁₈H₃₀N₆O₆ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (31 %, calc. one cyclisation per chain) and cyclotetrasarcosyl (0.8 %).

Cycloheptasarcosyl

Benzyloxycarbonyl(sarcosyl)₆sarcosine methyl ester (Z-Sar₇-OMe). Benzyloxycarbonyl(sarcosyl)₆sarcosine (3.3 g = 7.5 mmol), (sarcosyl)₂sarcosine methyl ester (1.9 g = 7.7 mmol) and dicyclohexylcarbodiimide (1.7 g = 8.25 mmol) in dichloromethane (70 ml) were reacted together

and worked up as described earlier to give a white powder (4.5 g = 90 %), TLC R_F 0.4 (D). 0.3 g was purified on a small silica gel column, m.p. 90 °C. (Found: C 53.99; H 7.06; N 14.47. $C_{30}H_{45}N_7O_{10}$ requires C 54.29; H 6.83; N 14.77).

Benzylloxycarbonyl(sarcosyl)₈sarcosine (Z-Sar₈-OH). Benzylloxycarbonyl(sarcosyl)₈sarcosine methyl ester (4 g = 6 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (6.3 ml) for 2 h and worked up as described for Z-Sar₈-OH. The resulting amorphous powder (3.8 g = 90 %) softened at 100 °C, homogeneous by TLC, R_F 0.3 (F).

Benzylloxycarbonyl(sarcosyl)₈sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₈-OTcp). Benzylloxycarbonyl(sarcosyl)₈sarcosine (2.2 g = 3.4 mmol), 2,4,5-trichlorophenol (1 g = 5.1 mmol) and dicyclohexylcarbodiimide (0.8 g = 3.9 mmol) in dichloromethane (70 ml) were reacted together and worked up in the usual way. The resulting white powder (2.5 g = 89 %) softened at 90 °C, TLC R_F 0.7 (D). 0.3 g was purified on a small silica gel column. (Found: C 51.06; H 5.35; $C_{35}H_{44}N_7O_{10}Cl_3$ requires C 50.70; H 5.35).

Cyclisation. Benzylloxycarbonyl(sarcosyl)₈sarcosine 2,4,5-trichlorophenyl ester (1.8 g = 2.2 mmol) in methanol (120 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.4 g) for 50 min and worked up to give a white solid (1.3 g = 1.57 mmol = 82 %) of the (sarcosyl)₈sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamide (20 ml) and added dropwise to stirred pyridine (600 ml) at 115 °C over a period of 70 min and stirred for 70 min more. After evaporation the residue was dissolved in methanol-water and passed through an ion-exchange column, eluted with methanol-water and evaporated. The residue was dissolved in acetone (5 ml) and kept in a refrigerator overnight. The crystals (300 mg) were collected, and consisted mainly of cycloheptasarcosyl, but with traces of cyclodi- and cyclotetrasarcosyl and some DC-urea. The impurities were removed from the cycloheptasarcosyl by sublimation (heated to 230 °C/0.01 mmHg). The filtrate was evaporated, treated with ether and sublimed. Yields of cyclic compounds: Cycloheptasarcosyl (38 %), sublim. temp. 280 °C/0.01 mmHg, m.p. 296 °C, TLC, R_F 0.4 (E) *m/e* 497. (Found: C 49.88; H 6.97; N 19.89. $C_{31}H_{33}N_7O_8$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (14 % calc. one cyclisation per chain) and cyclotetrasarcosyl (6 %).

Cyclooctasarcosyl

Benzylloxycarbonyl(sarcosyl)₈sarcosine methyl ester (Z-Sar₈-OMe). Benzylloxycarbonyl(sarcosyl)₈sarcosine methyl ester (9 g = 20 mmol) in methanol (350 ml) was hydrogenated over 5 % Pd-C for 3 h and worked up to give an oil

(6 g = 19 mmol = 95 %) of (sarcosyl)₈sarcosine methyl ester with an appropriate NMR spectrum. Without storing, the oil was dissolved together with benzylloxycarbonyl(sarcosyl)₈sarcosine (8 g = 18.3 mmol) in dichloromethane (200 ml) and dicyclohexylcarbodiimide (4.1 g = 20 mmol) added. The reaction conditions and work up was as usual, and resulted in a white powder (10 g = 80 %) m.p. 96 °C, TLC, R_F 0.45 (D). 0.3 g was purified on a small silica gel column. (Found: C 53.86; H 6.93; N 15.06. $C_{33}H_{40}N_8O_{11}$ requires C 53.94; H 6.86; N 15.25).

Benzylloxycarbonyl(sarcosyl)₈sarcosine (Z-Sar₈-OH). Benzylloxycarbonyl(sarcosyl)₈sarcosine methyl ester (4.5 g = 6.15 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (6.5 ml) for 2 h. A non-crystalline solid (3.6 g = 82 %) was isolated which softened at 90 °C, TLC, R_F 0.25 (F).

Benzylloxycarbonyl(sarcosyl)₈sarcosine 2,4,5-trichlorophenyl ester (Z-Sar₈-OTcp). From benzylloxycarbonyl(sarcosyl)₈sarcosine (1.8 g = 2.5 mmol), 2,4,5-trichlorophenol (0.8 g = 4 mmol) and dicyclohexylcarbodiimide (0.6 g = 2.9 mmol) in dichloromethane (40 ml) was isolated a white powder (1.9 g = 85 %) m.p. 90 °C, TLC, R_F 0.55 (D).

Cyclisation. Benzylloxycarbonyl(sarcosyl)₈sarcosine 2,4,5-trichlorophenyl ester (1.9 g = 2.1 mmol) in methanol (90 ml) and hydrochloric acid (1.0 ml) was hydrogenated over 5 % Pd-C for 50 min. A white solid (1.6 g = 2 mmol = 94 %) of (sarcosyl)₈sarcosine 2,4,5-trichlorophenyl ester hydrochloride was isolated. This was dissolved in dimethylformamide (50 ml), added dropwise to stirred pyridine (500 ml) at 115 °C over a period of 30 min, stirred for 1½ h more and evaporated. The residue was taken into methanol (10 ml) and undissolved material filtered off (550 mg) which appeared to be pure cyclooctasarcosyl. The filtrate was ion-exchanged in methanol, evaporated and the residue taken into methanol (10 ml). More cyclic octapeptide, together with some DC-urea was filtered off (30 mg) and purified by sublimation (heated until the DC-urea had sublimed, 220 °C). The filtrate was evaporated, treated with ether and sublimed, only a few mg of cyclooctasarcosyl was left. Yields of cyclic compounds: Cyclooctasarcosyl (55 %) sublim. temp. 280 °C/0.01 mmHg, m.p. 338 °C, TLC, R_F 0.4 (E), *m/e* 568. (Found: C 49.90; H 6.88; N 19.92. $C_{34}H_{40}N_8O_8$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (22 %) and cyclotetrasarcosyl (0.35 %). The yields are in both cases calc. from one cyclisation per chain.

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