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Cyclic Tetra- and Octapeptides of Sarcosine in Combination with Alanine or Glycine. Syntheses and Conformation

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A series of cyclic tetrapeptides, c-L-MeAla-Sar_3, c-L-Ala-Sar_3, c-D-Ala-Sar-L-Ala-Sar, c-D-Ala-Sar-D-Ala-Sar, c-L-Ala_2-Sar_2, c-Ala_3-Sar, c-Ala_4, c-Gly-Sar_3, c-Gly-Sar-Gly-Sar, c-Gly_2-Sar_2, c-Gly_3-Sar, c-Gly_4 have been synthesised. The yield in the cyclisation step depended on the amino acid sequence in the linear peptide. Cyclic dipeptides, formed by cleavage of the peptide chain during the cyclisation reaction, partially racemised tetrapeptides and octapeptides were also isolated. c-D-Ala-Sar-D-Ala-Sar could be obtained only from peptides which contained both D- and L-alanine residues. Most of the cyclic tetrapeptides adopted the conformation of the parent compound, cyclotetrasarcosyl which is centrosymmetric with the amide sequence *cis,trans,cis,trans*. The same conformation was also found even when an NH amide bond must thereby become *cis*. When α-substituents make this conformation impossible and in trifluoroacetic acid other conformers are observed, but these have the same *cis,trans* sequence.

The barriers to inversion are very high when one or two N-CH_3 amide groups are *trans*, but considerably lower when the two *trans* positions are occupied by NH amide groups, due to the ability of the latter to rotate through the ring.

When dissolved at − 60 °C, the isolated cyclic octapeptides, c-(L-Ala-Sar_3)$_2$, c-(L-Ala_2-Sar_2)$_2$, c-(Gly-Sar_3)$_2$, c-(Gly_2-Sar_2)$_2$, except c-Gly_8, showed the same conformation as cyclooctasarcosyl with the amide sequence *cis,cis,trans,trans, cis,cis,trans,trans*. On heating the solutions, conformational changes took place analogous to those observed in a series of cyclic pentapeptides.

Only a few isolated investigations on cyclic tetrapeptides have been reported. The simplest representative, cyclo-tetraglycyl, was the first to be synthesised by Schwyzer in 1956 [1] and cyclo-glycyltri-L-alanyl and cyclo-tetra-L-alanyl were prepared on an insoluble polymer.[2] A partially N-methylated tetrapeptide, tentoxin, cyclo-N-methyl-L-alanyl-L-leucyl-N-methyl-*trans*-dehydrophenylalanylglycyl has been isolated [3] from a pathogenic fungus and studied by X-ray methods [4] and NMR spectroscopy.[5] NMR studies have also been carried out on three cyclic tetrapeptides of proline combined with either glycine or sarcosine.[6]

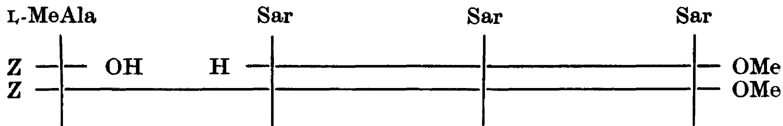
The simplest fully N-methylated cyclic tetrapeptide, cyclotetrasarcosyl, synthesised in this laboratory, shows a high degree of conformational homogeneity. The same centrosymmetric conformation with the amide sequence *cis,trans,cis,trans* (Fig. 6 A), was found both in solution [7,8] and in the crystal.[9] For c-Gly_4, a conformation with the four amide groups *trans* [1] has been suggested. Theoretical calculations on c-L-Ala_4 concluded that with all amide bonds *trans* an acceptable conformation could be obtained only if these were distorted from planarity.[10] It was therefore of interest to examine whether the unique conformation of c-Sar_4 might be adopted by other cyclic tetrapeptides and to study favoured conformations when *trans* preferred NH amides are present.

A series of cyclic tetrapeptides have been synthesised, replacing successively all four sarcosine units by either alanine (D and L) or glycine residues. (Scheme 1). Some results from the conformational study have already been communicated.[11,12] This paper describes the syntheses and gives additional results from the conformational investigations.

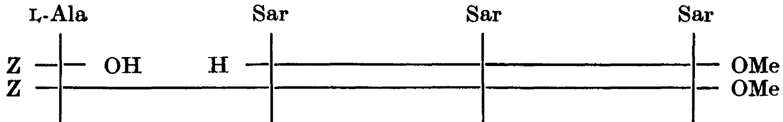
From some of the cyclisation reactions were also isolated cyclic octapeptides (Table 1) which represent an interesting extension of our investigations, and will be discussed.

Scheme. 1. Routes to the different linear tetrapeptides.

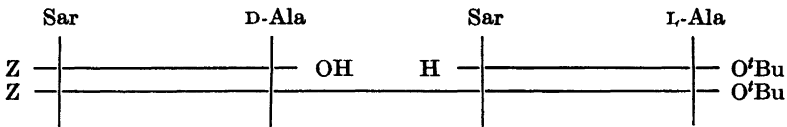
For cyclo-*N*-methyl-L-alanyltrisarcosyl, c-L-MeAla-Sar_3

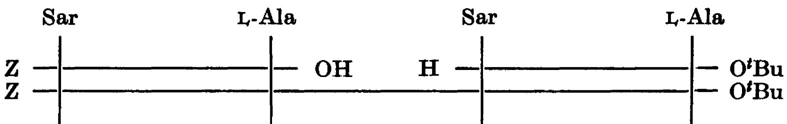


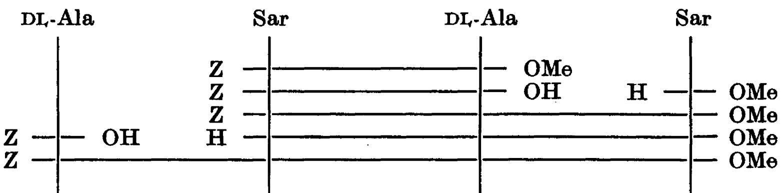
For cyclo-L-alanyltrisarcosyl, c-L-Ala-Sar_3



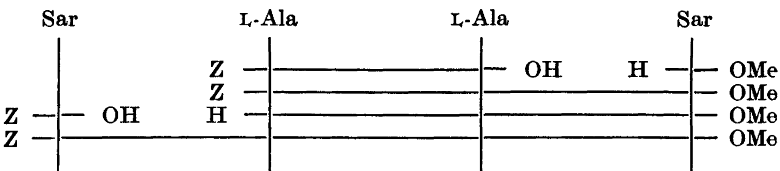
For cylco-D-alanylsarcosyl-L-alanylsarcosyl, c-D-Ala-Sar-L-Ala-Sar
and cyclo-D-alanylsarcosyl-D-alanylsarcosyl, c-D-Ala-Sar-D-Ala-Sar



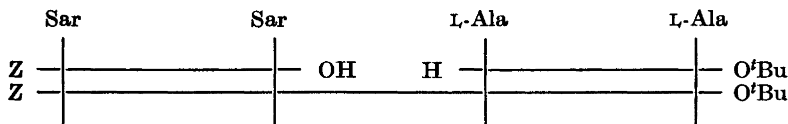




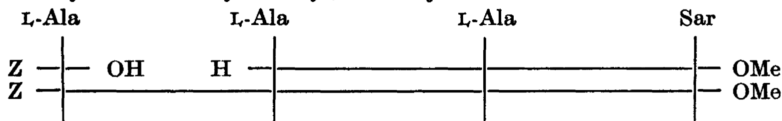
For cyclo-di-L-alanyldisarcosyl, c-L-Ala_2-Sar_2



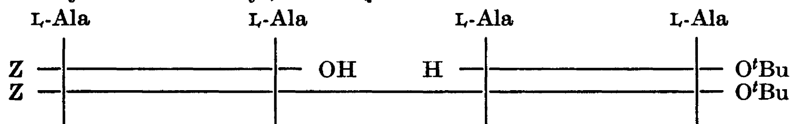
Scheme 1. Continued.



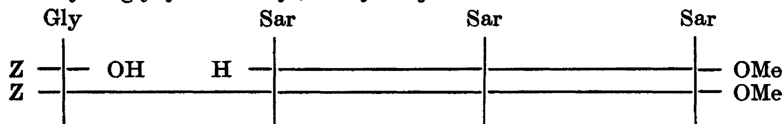
For cyclo-tri-L-alanylsarcosyl, c-L-Ala_3 Sar



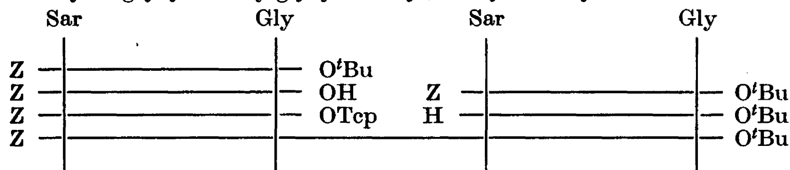
For cyclo-tetra-L-alanyl, c-L-Ala_4



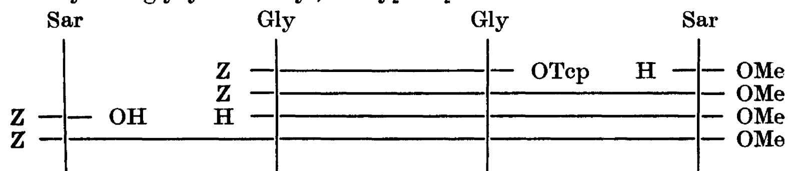
For cyclo-glycyltrisarcosyl, c-Gly-Sar_3

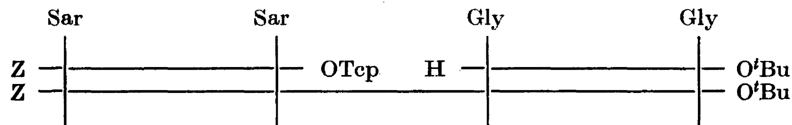


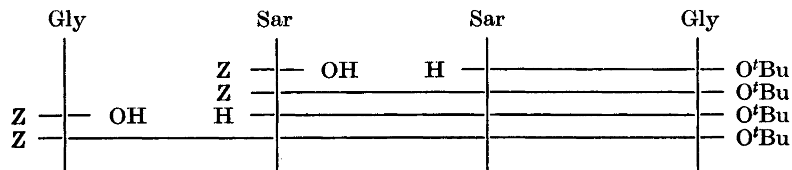
For cyclo-glycylsarcosylglycylsarcosyl, c-Gly-Sar-Gly-Sar



For cyclo-diglycyldisarcosyl, c-Gly_2-Sar_2

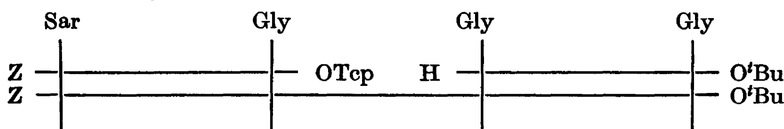






Scheme 1. Continued.

For cyclo-triglycylsarcosyl, c-Gly$_3$-Sar



For cyclo-tetraglycyl, c-Gly$_4$

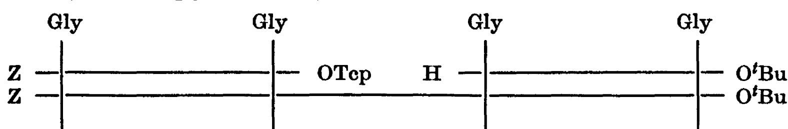


Table 1. Yields (%) of cyclic peptides from tetrapeptide trichlorophenyl ester.

Starting material	Dipeptides		Tetrapeptides		Octapeptides	
HCl.L-MeAla-Sar$_3$-OTcp			c-L-MeAla-Sar$_3$	(20)	c-(L-MeAla-Sar$_3$)$_2$	(traces)
HCl.L-Ala-Sar$_3$-OTcp	c-Sar$_2$	(33)	c-Ala-Sar$_3$	(25)	c-(Ala-Sar$_3$)$_2$	(traces)
	c-Ala-Sar	(16)				
HCl.Sar-D-Ala-Sar-L-Ala-OTcp			c-D-Ala-Sar-L-Ala-Sar	(27)		
			c-D-Ala-Sar-D-Ala-Sar	(1)		
HCl.Sar-L-Ala-Sar-L-Ala-OTcp			c-D-Ala-Sar-L-Ala-Sar	(1)		
			c-L-Ala-Sar-L-Ala-Sar	(traces)		
HCl.L-Ala-Sar-L-Ala-Sar-OTcp	c-Ala-Sar	(64)	c-D-Ala-Sar-L-Ala-Sar	(<1)		
HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp	c-Ala-Sar	(52)	c-D-Ala-Sar-L-Ala-Sar	(5)		
			c-D-Ala-Sar-L-Ala-Sar c-L-Ala-Sar-L-Ala-Sar	(4)		
HCl.Sar-L-Ala$_2$-Sar-OTcp			c-L-Ala$_2$-Sar$_2$	(2.5)	c-(L-Ala$_2$-Sar$_2$)$_2$	(3)
HCl.Sar$_2$-L-Ala$_2$-OTcp	c-Sar$_2$	(30)				
	c-Ala$_2$	(13)				
HCl.L-Ala$_3$-Sar-OTcp			c-Ala$_3$-Sar	(4.5)		
HCl.L-Ala$_4$-OTcp			c-Ala$_4$	(5)		
HCl.Gly-Sar$_3$-OTcp	c-Gly-Sar	(6)	c-Gly-Sar$_3$	(23)	c-(Gly-Sar$_3$)$_2$	(3)
	c-Sar$_2$	(4)				
HCl.Sar-Gly-Sar-Gly-OTcp			c-Gly-Sar-Gly-Sar	(42)		
HCl.Sar-Gly$_2$-Sar-OTcp			c-Gly$_2$-Sar$_2$	(3)	c-(Gly$_2$-Sar$_2$)$_2$	(13)
HCl.Sar$_2$-Gly$_2$-OTcp	c-Sar$_2$	(25)	c-Gly$_2$-Sar$_2$	(traces)	c-(Gly$_2$-Sar$_2$)$_2$	(3)
	c-Gly$_2$	(10)				
HCl.Gly-Sar$_2$-Gly-OTcp	c-Gly-Sar	(57)				
HCl.Sar-Gly$_3$-OTcp			c-Gly$_3$-Sar	(3)		
HBr.Gly$_4$-SCH$_2$COOH			c-Gly$_4$	(4.5)	c-Gly$_8$	(3)

SYNTHESES

The linear peptides were synthesised as shown in Scheme 1. The benzyloxycarbonyl group was used for protection of the amino function, the methyl ester or *t*-butyl ester for the carboxyl group and *N*,*N*′-dicyclohexylcarbodiimide as the coupling reagent. For cyclisation the methyl (*t*-butyl) ester was first hydrolysed, the carboxyl group then converted to the 2,4,5-trichlorophenyl ester, and the

peptide active ester after removal of the benzyloxycarbonyl group cyclised in pyridine at 115 °C. The cyclic peptides were isolated either by fractional crystallisation, sublimation, or chromatography on silica gel.

Some tetrapeptides gave the corresponding cyclic peptides in high yield (40 %), while closely related peptides produced the cyclic tetrapeptides either in low yield or not at all; instead cyclic dipeptides, octapeptides and racemised tetrapeptides were formed (Table 1). The product distribution clearly demonstrated that the sequence of amino acids in the linear tetrapeptide is important. The yield of cyclic tetrapeptide being high when the linear peptide has a high probability of being folded in the same way as the strongly preferred conformation of c-Sar_4 (Fig. 6A) which has alternating *cis* and *trans* amide bonds and requires the configuration DDLL (or LLDD) of the amino acids. This means that a suitable sequence consists of alternating *N*-methylated amino acids, such as sarcosine, which easily form *cis* amide bonds. In addition, it was found preferable to have an NH amino acid as the second residue in the chain (see below).

The major byproduct was formation of cyclic dipeptides and the product distribution (Table 1) corresponds to cleavage of the central amide bond and shows that the reaction takes place in the linear peptide. If the second amino acid is *N*-methylated the free amino end can easily move close to the second carbonyl carbon and form an unstable cyclol.[13,14] This splits up to give a cyclic dipeptide and a dipeptide active ester which subsequently may cyclise to a second molecule of cyclic dipeptide. This reaction is dominating when the fourth amino acid is of the *trans*-preferred NH-type (alanine or glycine), whereas if the fourth amino acid is *N*-methylated formation of the cyclic tetrapeptide is preferred to formation of the unstable cyclol. Hardly any cyclic dipeptides are formed when the second amino acid is alanine or glycine, which was necessary to have to obtain c-L-Ala_2-Sar_2 and c-Gly_2-Sar_2 (Table 1).

Doubling reactions, well-known from cyclisation of tri- and pentapeptides[15] and also encountered in tetradepsipeptides,[16,17] was found to take place, but to little extent, in the cyclic homologues of sarcosine,[8] and the cyclic pentapeptides of sarcosine combined with either alanine or glycine.[18] In the present tetrapeptides, a few of the reactions led to isolable quantities of cyclic octapeptides (Table 1). Highest yield (13 %) was obtained on cyclising Sar-Gly_2-Sar-OTcp, which gave a low yield of cyclic tetrapeptide and cannot easily form cyclic dipeptides.

Racemisation has been shown to occur during cyclisation of pentapeptides.[18] Also from the cyclisation of tetrapeptides, pairs of cyclic tetrapeptides were isolated (Table 1). Sar-D-Ala-Sar-L-Ala-OTcp gave two cyclic tetrapeptides which were isolated either by fractional crystallisation or by sublimation. They had different IR and NMR spectra, but identical mass spectra (*m/e* 284). The NMR spectrum of the major product clearly showed this to be c-D-Ala-Sar-L-Ala-Sar with the same conformation as c-Sar_4, centrosymmetric and with the alanine residue in the *trans* positions. The other compound showed the presence of two conformers (in the NMR spectrum) and had a high optical rotation ($[\alpha]_D + 92°$) and must be c-D-Ala-Sar-D-Ala-Sar, formed by racemisation of the C-terminal L-alanine. The peptide chain has a high probability of being folded in the same way as the favoured conformation of the major product and the free amino end can easily approach the active ester and form an intermediate as shown in Fig. 1. Cleavage of the active ester may occur by elimination of phenol removing either H from the amino group which will result in the major product or by elimination of the α-H of

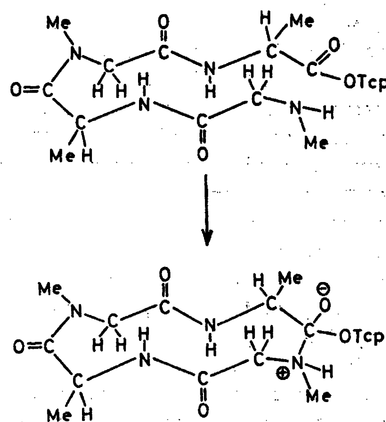


Fig. 1.

Table 2. Properties of the

	Sub-lim. temp. °C/0.01 mmHg	M.p.,°C	IR (cm^{-1}) bands in KBr		Low temp. NMR data (100 MHz)				
			C=O stretch	*trans* CONH	NMR coal.-temp. °C	Chem. shift, ppm (gem. coupl. *C*-CH_3 *trans*	*C*-CH_3 *cis*	*N*-CH_3 *trans*	*N*-CH_3 *cis*
c-L-MeAla-Sar_3	180	318	1670,1640			1.26		2.66,2.87	3.04
c-L-Ala-Sar_3	190	315(subl.)	1690,1680,1650	1550		1.28		2.83	3.03,3.07
c-D-Ala-Sar-L-Ala-Sar	290	> 350	1670,1650	1570		1.41			3.23
c-D-Ala-Sar-D-Ala-Sar	220	340(subl.)	1670,1650,1640	1550	30	1.33,1.42			3.03,3.08
c-L-Ala_2-Sar_2	240	310(subl.)	1670,1640	1570		1.42	1.62	3.09	3.21
c-Ala_3-Sar	250	> 350	1660	1560		1.39,1.40	1.57		3.25
c-Ala_4	210	315(subl.)	1650	1540		complex			
c-Gly-Sar_3	230	322	1685,1650	1550	150			2.85	3.02,3.08
c-Gly-Sar-Gly-Sar	260	> 350	1660	1560	30				3.17,3.20
c-Gly_2-Sar_2	260	310	1690,1670,1650	1560	100			2.89,3.02	3.09
c-Gly_3-Sar	270	> 350	1670,1650,1640	1540					3.26
c-Gly_4(subl.)	270	340(subl.)	1690,1640	1540					
c-Gly_4 (cryst.H_2O)		340(subl.)	1680,1660	1550					

the L-alanine residue which subsequently will give the two obtained cyclic tetrapeptides. When Sar-L-Ala-Sar-L-Ala-OTcp and L-Ala-Sar-L-Ala-Sar-OTcp which cannot easily fold in the same way as c-Sar_4 were cyclised only traces of cyclic tetrapeptides (LL and DL-isomers) were isolated, and c-Ala-Sar was the major product (64 %) from the latter peptide. When DL-Ala-Sar-DL-Ala-Sar-OTcp was cyclised, besides some cyclic dipeptides, both c-D-Ala-Sar-L-Ala-Sar (5 %) and the DD + LL isomer (4 %) were isolated. These experiments show that the DD- and LL-isomers of c-Ala-Sar-Ala-Sar are obtained only through racemisation of peptides containing both D- and L-alanine residues. The c-Ala_3-Sar was isolated in low yield and adopted the c-Sar_4 conformation, as shown by NMR spectroscopy. The expected c-L-Ala_3-Sar (Table 1) cannot adopt this conformation, and some racemisation must have taken place possibly in the coupling reactions since DCC was used. Similarly the X-ray analysis of the expected c-L-Ala-Sar_3 showed that the crystal contained the racemate.[19]

This demonstrates that favoured conformations of the peptide chains play an important role both for ring size and yield of the resulting cyclic peptides.

Physical and spectral properties of the cyclic tetrapeptides

The cyclic tetrapeptides are well defined solids, sublimable and with sharp melting points (Table 2). Their solubility in organic solvents is generally low, lower than that of the corresponding cyclic octapeptides and the closely related cyclic pentapeptides.[18] A few are even only slightly soluble in water. The mass spectra show the molecular ions as intense peaks. The fragmentation patterns are specific for each cyclic peptide indicating that they split up differently depending on the amino acids present. This is in contrast to the

cyclic tetrapeptides.

const., Hz) of CH_2: C-2 & C-8 outer	inner	C-5 & C-11 outer	inner	CH	NH	Solvent
3.23	5.3 (14.5)	3.54,3.58	4.33,4.41(18)	5.4		$CDCl_3$
3.24	5.39(14.5)	3.54,3.58	4.14,4.38(18)	4.86	6.88(10)	$CDCl_3$
		4.03	4.39 (18)	5.13	7.62(10)	TFA
		3.75,3.85,3.90	3.99,4.31,4.55(18)	4.95	7.49(10),7.68(7),8.01(10)	$CDCl_3$ trace TFA
3.6	5.6 (15)	4.08	4.68(18)	4.34,5.12	7.78(10),7.92(8)	TFA
		4.02	4.38(18)	4.24,5.11,5.11	7.63(10),7.81(10),7.83(7)	TFA
3.44,3.53	5.25,4.84(15)	3.69,3.70	4.14,4.32(18)		6.86(10)	$CDCl_3$ trace TFA
3.83	5.13(15)	4.10	4.48(18)		7.61(10),7.94	TFA
3.47,3.54	4.85,5.28(15)	3.81	4.18(18)			$CDCl_3$ trace TFA
3.92	5.30(15)	not resolved			8.22 8.78	TFA
		4.07(7)	4.19(9)		7.91	TFA

behaviour of the cyclic pentapeptides [18] which showed identical fragmentation patterns. The infrared spectra show from two to four carbonyl absorptions (Table 2) but it was not possible to assign these to *cis* and *trans* amide bonds or to particular locations in the molecules. The cyclic tetrapeptides which contain one or more NH-amide bonds always show the diagnostic *trans*-CO−NH band at 1550 cm^{-1}. An indication of the number of *trans* amide bonds could be obtained by comparing the ratio of the area below this NH-absorption and the CO-absorptions. The NMR spectra which mostly could be recorded below the coalescence of the rings showed that only one conformation is present in $CDCl_3$, while additional conformers were observed in TFA or mixtures of $CDCl_3$ and TFA. Dropwise addition of C_6D_6 was useful to resolve overlapping signals and also to determine conformations due to the different upfield shifts of the lines.

THE INDIVIDUAL CYCLIC TETRAPEPTIDES

A few crystal structures, c-Ala-Sar_3, c-Gly-Sar_3 and c-Gly-Sar-Gly-Sar have been determined by X-ray methods [19] and a conformation identical to that of c-Sar_4 [9] is adopted with the same dihedral angles and transannular distances and with the NH amide groups in the *trans* positions.

In solution ($CDCl_3$) the NMR spectra (Table 2) of c-L-MeAla-Sar_3, c-L-Ala-Sar_3 [11] and c-Gly-Sar_3 are closely related to that of c-Sar_4 which has two N-CH_3 singlets *cis* and *trans* (at highest field) and two methylene quartets with J of 18 and 14 Hz (most widely separated). The same conformation is adopted and the NH amide group of the two latter occupies a *trans* position shown in IR by the *trans*-CO-NH band at 1550 cm^{-1}. The α-CH_3 groups are situated in the 2-(or 8-)position (Fig. 6 A), necessarily pointing out of the ring. This position in

c-Sar_4 is bent more out of the ring than the corresponding 5-(or 11-) position and explains why the α-CH_3 group of c-L-MeAla-Sar_3 prefers the 2-position. The NMR signals may now be correlated with the different protons in the molecules. The CH_2 quartet with $J=14$ Hz belongs to the 2(8)-position with the inner H shifted to low field. The N-CH_3 singlets at lowest field belong to the *cis* amide bonds. This is opposite to what has been found in linear peptides such as polysarcosine [20] and poly-N-methyl-L-alanine.[21] The dihedral angles of NHCH correspond well with the observed coupling constants, the inner H having a $J=10$ Hz and the high-field H (the outer proton) a small coupling constant. According to the Bystrov curve,[22] large values are expected for angles of 0 and 180° and small values for angles around 60°. The geminal coupling constants (18 and 14 Hz) fit the reported dependence [23] on the orientation of the methylene group to an adjacent carbonyl group, being largest when the dihedral angle of $R-C_{CO}-C_{\alpha}-R'$ is 0 or 180° and smallest for 60 and 120°. In TFA, the NMR spectra of c-L-Ala-Sar_3 and c-Gly-Sar_3 (Fig. 2) show that two conformers are present, the major is identical to that in $CDCl_3$, the minor, 20 and 30 % (Fig. 2a) respectively, has the same large (10 Hz) and small NHCH coupling and the same CH_2-quartets ($J=18$ and 15 Hz). A noteworthy difference is that the lowest shifted CH_2-proton (Fig. 2) (at δ 5.4) is now situated at δ 4.6. The N-CH_3 lines appear unresolved in the *cis* N-CH_3 region, but on dropwise addition of benzene one moves faster

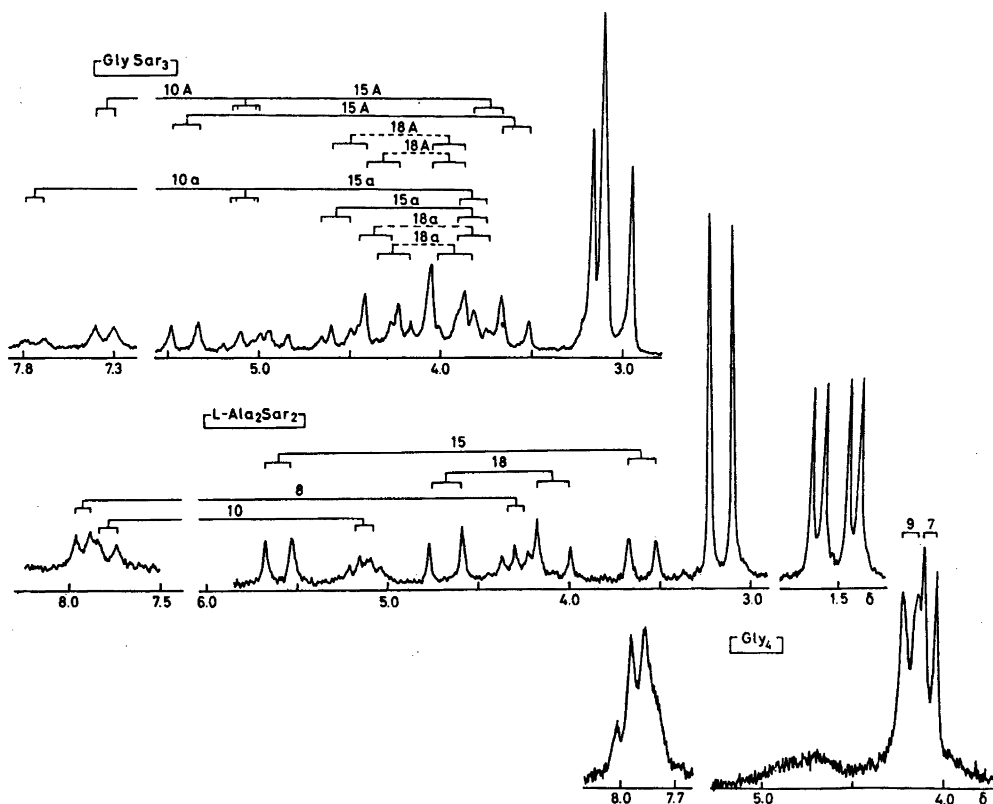


Fig. 2. The 100 MHz NMR spectrum at 35 °C of c-Gly-Sar_3 in TFA:$CDCl_3$ = 4:1 (upper spectrum). A represents the major conformer and a the minor conformer, c-L-Ala_2-Sar_2 in TFA and c-Gly_4 in TFA (lower spectrum). The splitting pattern was partially deduced from spin-decoupling and the quartets shown by dotted lines are presumed from the C_6D_6 addition.

to higher field which indicates that this belongs to a *trans* amide bond.[18] This conformation will be considered in the discussion.

c-D-Ala-Sar-L-Ala-Sar shows in TFA (the only solvent which could be used) an NMR spectrum [12] (Table 2) which fits the c-Sar_4 conformation with the NH-amide bonds *trans*. The rate of exchange of the amide protons is particularly slow in this molecule, full exchange in TFA-*d* takes more than 12 h at 25 °C, whereas in both c-Ala-Sar_3 and c-Gly-Sar_3 full exchange take less than 5 min. This may suggest that a second conformer is not present in c-D-Ala-Sar-L-Ala-Sar and that the rate of exchange of the *trans* amide protons is generally slow in the c-Sar_4 conformation.

c-Gly-Sar-Gly-Sar shows in TFA (the only solvent which could be used) an NMR spectrum at −20 °C with two conformers present in equal concentrations whereof one is the crystal conformation. The second conformer is unsymmetric with two hardly split N-CH_3 singlets (seen when C_6D_6 is added), a broadened NH line and the CH_2-protons are broad and unresolved and did not sharpen on further cooling to −30 °C. Addition of benzene moved the N-CH_3 lines only slightly to higher field indicating that they belong to *cis* amide bonds. At about −10 °C the two N-CH_3 singlets of the unsymmetric conformer coalesced to a single line and the CH_2-protons appeared as one broad line. No change took place in the conformer adopting the c-Sar_4 skeleton until 20 °C, then the CH_2-quartets started to broaden and at 70 °C, the NMR spectrum shows only two sharp lines of the CH_2-protons and one N-CH_3 singlet. The conformational situation will be considered in the discussion.

c-L-Ala_2-Sar_2 (Fig. 2) shows with its two α-CH_3 doublets, two N-CH_3 singlets, two CH_2-quartets, $J = 18$ and 15 Hz two CH bands and two NH doublets, $J = 10$ Hz (slowly exchanged with D) and 8 Hz (rapidly exchanged with D), that the conformation of c-Sar_4 is adopted having one N-CH_3- and one NH-amide group *trans* and the second N-CH_3- and NH-amide group *cis*. This clearly demonstrates that the conformation with alternating *cis* and *trans* amide bonds is retained even when a *trans* preferred NH-amide bond has to be *cis*. Any additional lines due to a second conformer was not visible in the NMR spectrum.

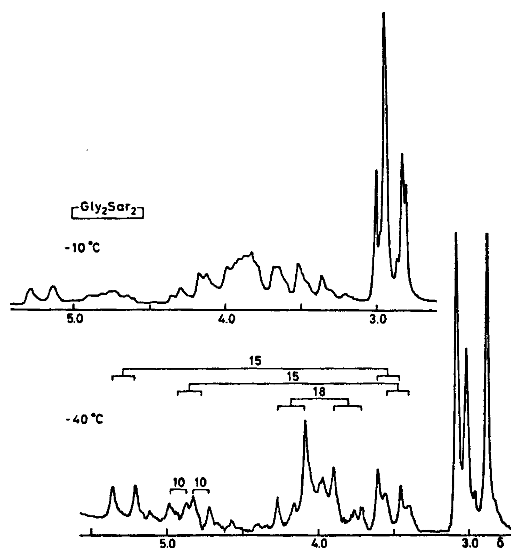


Fig. 3. The 100 MHz spectrum of c-Gly_2-Sar_2 at −40 °C and at −10 °C (upper spectrum). Crystals were dissolved in $CHFCl_2$ with a trace of TFA at −50 °C and allowed to warm up in the probe (NH region left out).

The closely related c-Gly_2-Sar_2 shows when dissolved in $CHFCl_2$ (added traces of TFA) at −40 °C (Fig. 3) one major conformer (*ca.* 80 %) with two N-CH_3 singlets, one not resolved CH_2-quartet with $J = 18$ Hz and two with $J = 15$ Hz whereof one exhibits a large ($J = 10$ Hz) and a small vicinal coupling. The other CH_2 group is not well resolved, but the quartet is narrow. The NH protons could not be observed in this solvent. The NMR spectrum of the major conformer shows that the c-Sar_4 conformation is adopted with one N-CH_3- and one NH-amide *cis* and the other N-CH_3- and NH-amide *trans*. Two possibilities exist for arranging the groups around the ring (Fig. 6 A); the two NH groups can be either in positions 1 and 4 or in 4 and 7, only the former is allowed for c-L-Ala_2-Sar_2 due to α-substituents. The NMR data do not decide which of the two is adopted by c-Gly_2-Sar_2 at low temperature and thus in the solid. The additional conformer increases in concentration on raising the temperature and at the thermodynamic equilibrium (*ca.* −10 °C) (Fig. 3) this conformer is dominating, about 20 % of the original one remains and a few extra N-CH_3 lines may belong to a third conformer possibly the other allowed

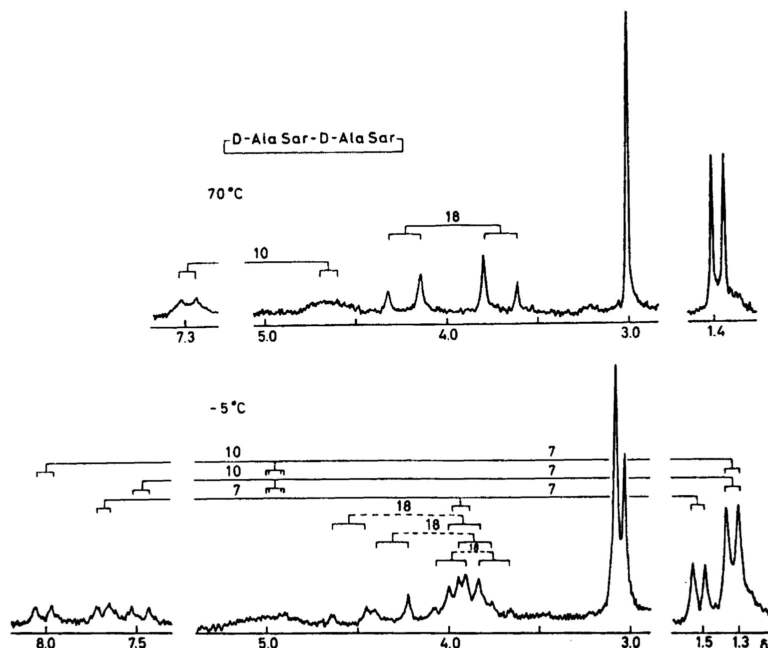


Fig. 4. The 100 MHz NMR spectrum of c-D-Ala-Sar-D-Ala-Sar in $CDCl_3$ plus a trace of TFA at 70 and −5 °C (lower spectrum). The splitting pattern was partially deduced from spin-decoupling and the quartets shown by dotted lines are presumed from the C_6D_6 addition.

on the c-Sar_4 skeleton. The second conformer has the N-CH_3 lines unresolved in the *cis* N-CH_3 region and one moves fast to higher field on addition of C_6D_6 (to a $CDCl_3$ solution). The signals of the methylene protons are unresolved and broad ($CDCl_3$ solution) but sharpen on cooling to −20 °C suggesting a coalescence phenomenon for this conformer. The CH_2-quartets of the original conformer do not coalesce below 80 °C, a similar phenomenon as observed in c-Gly-Sar-Gly-Sar where the conformer which adopts the c-Sar_4 conformation has a high barrier to inversion whereas the second exhibits a much lower barrier to inversion (see the discussion).

c-D-Ala-Sar-D-Ala-Sar shows at 70 °C a simple NMR spectrum (Fig. 4) (one N-CH_3 singlet, one CH_2-quartet, $J = 18$ Hz, and one NH doublet, $J = 10$ Hz). On cooling, the quartet began to broaden and at 5 °C (Fig. 4) two species (2:1) are present, the minor has the same lines as the spectrum recorded at 70 °C. The major conformer is unsymmetric with two N-CH_3 lines, two not well resolved CH_2-quartets which both seem to have $J = 18$ Hz and two NH doublets, $J = 10$ and 7 Hz. The ratio of the conformers did not change on changing the solvent from TFA to $CDCl_3$ (with a trace of TFA). This molecule cannot adopt the c-Sar_4 conformation due to the configuration of the alanine residues and alternative conformations will be considered in the discussion.

c-Ala_3-Sar was isolated in low yield and shows an NMR spectrum (in TFA) (Table 2) which by comparison with the NMR spectrum of c-L-Ala_2-Sar_2 fits exactly this conformation having two NH-amide groups *trans* and one *cis*. The expected c-L-Ala_3-Sar cannot adopt the c-Sar_4 conformation due to the α-substituents and some racemisation must have taken place. The isolated peptide has to be either c-L-Ala_2-D-Ala-Sar or c-D-Ala_2-L-Ala-Sar the only possible on the c-Sar_4 skeleton.

c-Gly_3-Sar shows at −25 °C (in TFA with a trace of $CDCl_3$) one N-CH_3 singlet, one CH_2-quartet ($J = 15$ Hz) which exhibits a large (10 Hz) and a small CHNH coupling. The

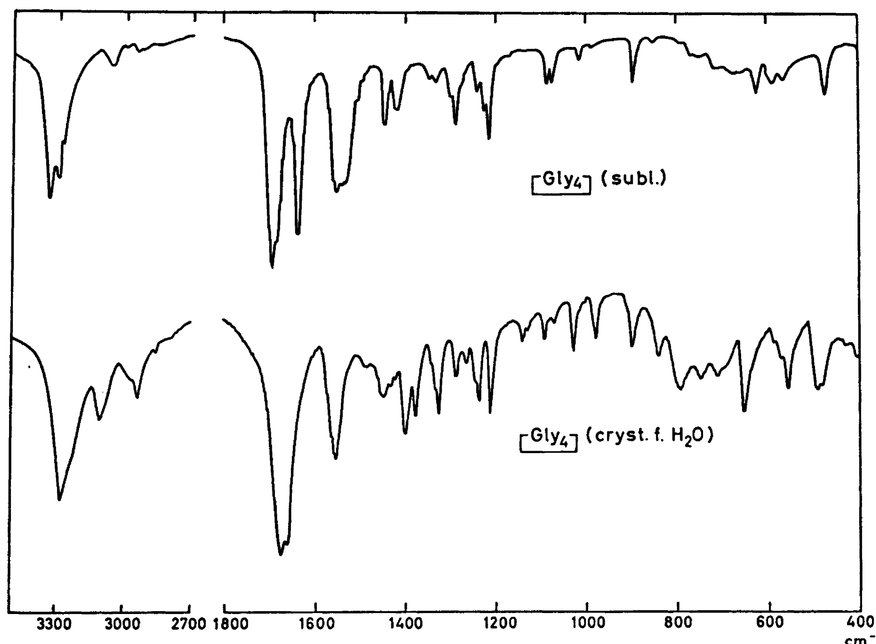


Fig. 5. The IR spectrum of c-Gly$_4$ as a KBr-disc, crystallised from water (lower curve) and sublimed.

remaining CH_2 protons are unresolved (at δ 4.35) and the NH protons are grouped at δ 8.2 (broadest) and 8.7. On warming, the CH_2 lines start to coalesce at -15 °C, and addition of benzene moves the *N*-CH_3 group comparatively little upfield which indicates that this is part of a *cis* amide bond. No certain conclusion can be drawn for this molecule.

c-L-Ala$_4$ was obtained in low yield and shows a complex NMR spectrum (in TFA) with more than eight α-CH_3 doublets indicating the presence of either several conformers or isomers containing both D- and L-alanine residues due to racemisation during the synthesis. c-L-Ala$_4$ with all amide groups *trans* should have only one α-CH_3 doublet.

c-Gly$_4$ crystallised from water as very small crystals which were insoluble in organic solvents and only slightly soluble in water and TFA and it was difficult to dissolve enough for the NMR measurements. The crystallised product could be sublimed (at 270 °C/0.01 mmHg) and thereby became a little more soluble in TFA. The sublimed product could on the other hand not easily be resublimed as a higher temperature was now required. The IR spectra (in KBr) (Fig. 5) of the crystallised and sublimed c-Gly$_4$ are different. Among several dissimilarities is the ratio of the area below the NH-absorption at 1550 cm^{-1} and the CO-absorptions at 1640–1690 cm^{-1} (NH/CO), being smallest for the crystallised product. Comparison with this NH/CO ratio of the other cyclic tetrapeptides of known conformation suggests that the crystallised c-Gly$_4$ contains two *trans* amide bonds while the sublimed product contains three or four *trans* amide bonds. This ratio may vary with alternation of the conformation even without change in the number of *trans* amide bonds, although it is true, c-Gly$_6$ and c-Gly$_8$ which have all amide bonds *trans* showed a higher ratio. Only one of the other cyclic tetrapeptides, c-Ala$_4$, isolated by sublimation only, showed the same high NH/CO ratio as the sublimed c-Gly$_4$ and could possible have an analogous behaviour. Such a difference was not observed for the sarcosine containing cyclic tetrapeptides. c-Gly-Sar-Gly-Sar, for instance, was crystallised from water and also sublimed but showed no spectral (IR and NMR) differences. A slow conversion of the sublimed c-Gly$_4$ to the crystallised c-Gly$_4$ was observed as a KBr-disc of sublimed product after one

year gave an IR spectrum showing the presence of both conformations. A fast conversion took place on dissolution (in TFA) of the sublimed product as the IR spectrum of the evaporated sample was identical to that of the crystallised product. The NMR spectra (in TFA) of crystallised and sublimed (Fig. 2) c-Gly$_4$ are identical showing two partly resolved NH triplets and two CH_2 doublets, one, ($J = 9$ Hz) is broader than the other ($J = 7$ Hz). Addition of benzene moved the broadest doublet most upfield, 84 Hz and 65 Hz (1 ml C_6D_6 added to 0.5 ml TFA). By cooling to -20 °C the peaks became broad and unresolved but did not change on further cooling to -50 °C (traces of $CDCl_3$ was added). This NMR spectrum can only fit conformations with alternating *cis* and *trans* amide bonds and with the methylene groups rapidly flipping. If the four amide bonds were *trans* as originally suggested by Schwyzer [1] only one CH_2 doublet should be present. A recent reinvestigation using NMR (^1H and ^{13}C) spectroscopy [25] also led to the conclusion that this molecule has four *trans* but not planar, amide bonds. This is in disagreement with our findings, but may perhaps be explained by postulating that these authors have studied the doubling product, c-Gly$_8$, which may sometimes be obtained in their synthesis (see Experimental part).

DISCUSSION

The conformation of c-Sar$_4$ (Fig. 6 A) is obviously very favoured in cyclic tetrapeptides as it is chosen by most of the cyclic tetrapeptides in this series both in the solid and in solution and also by cyclic peptides where NH amide bonds have to be *cis*. A crystal structure of dehydrotentoxin [4] showed the same conformation even with the two NH amide bonds *cis* and the *N*-CH_3 amide bonds *trans*, the only possibility on this skeleton due to the α-substituents. The crystal structure of three closely related tetradepsipeptides showed that one, c-D-MeVal-L-Hyv-L-MeVal-D-Hyv,[26] adopts an almost identical conformation to c-Sar$_4$ with the ester groups in the *trans* positions, whereas the DDDL-isomer and the c-L-MeLeu-D-Hyv-L-MeIle-D-Hyv [27] which cannot adopt this conformation because of the relative configuration of the α-substituents, choose a conformation having also alternating *cis* amide and *trans* ester bonds but with the four carbonyl oxygens on the same side of the molecule. A difference exists, however, between the depsipeptide and the peptide structures, the ester oxygens are bent towards the transannular carbonyl carbon while in the peptides both the *trans* *N*-CH_3 (NH) and the CO groups are more perpendicular to the ring. No crysta

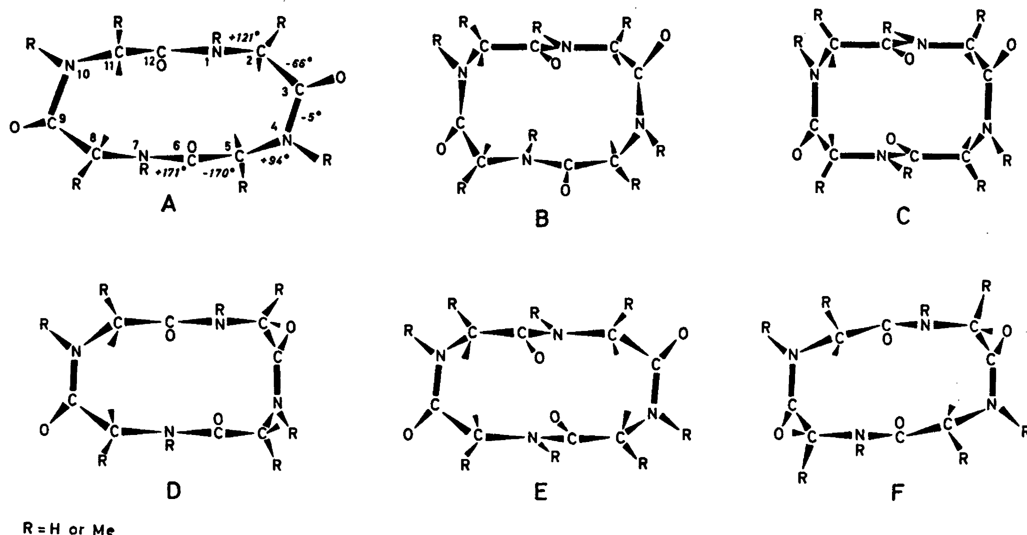


Fig. 6. Possible conformations.

structures have yet been determined of cyclic tetrapeptides which do not adopt the c-Sar_4 conformation. In solution a few proline containing cyclic tetrapeptides have been studied by NMR (^1H and ^{13}C) spectroscopy,[6] and the alternating *cis,trans* amide sequence is evidenced. In our series, c-D-Ala-Sar-D-Ala-Sar does not adopt the c-Sar_4 conformation and additional conformers are observed in TFA for some of the cyclic tetrapeptides. Since only the amide sequence *cis,trans,cis,trans* is observed with certainty in such twelve membered rings, conformations with this sequence only will be considered here.

The five possible conformations (Fig. 6 B–F) can be derived from the c-Sar_4 conformation (Fig. 6 A) by the following operations.* Rotation by 180° of one *trans* amide group leads to conformer B and rotation of both to C. Flipping of one and both *cis* amide groups also turn the adjacent methylene groups and lead to D and C, respectively. Conformer E is deduced from c-Sar_4 by flipping of one *cis* and rotation of one *trans* amide group and conformer F by flipping of the same *cis* amide group and rotation of the other *trans* amide group. Both possess two-fold axial symmetry and require the LLLL (DDDD) configuration of the amino acids. Conformer A and C are centrosymmetric and require the configuration DDLL while B and D are unsymmetric and require the configurations DDLL and DDDD (LLLL), respectively. When the ring contains two *trans* NH-amide groups, rotation of these through the ring exhibit a low barrier; thus, *ca.* 14 kcal/mol ($T_c = 30$ °C) is found for c-Gly-Sar-Gly-Sar.** With one or two *trans* N-CH_3 amide bonds present, as in c-Gly-Sar_3 and c-Sar_4, the coalescence temperatures are high 150 °C (Table 2) and 180 °C,[8] respectively and correspond to inversion barriers of *ca.* 21 and 24 kcal/mol. Rotation of a *trans* amide group through the ring is then so hindered that full inversion may be obtained through a different process.[24] Flipping of the *cis* amide groups should require low energy since no group has to rotate through the ring.

c-D-Ala-Sar-D-Ala-Sar shows one major unsymmetric and one minor symmetric conformer (Fig. 4) whereof the unsymmetric conformer with its coupling constants (10, 7 and 16–18 Hz) fits the conformer D (Fig. 6) with the NH-amide groups *trans*. The symmetric one may either be conformer E or F (Fig. 6), both can be derived from D by rotation of one *trans* NH-amide bond which requires relatively low energy and corresponds to the observed coalescence (30 °C). The coupling constants ($J = 10$ and 18 Hz) do not decide whether conformer E or F is preferred, but may indicate that it is F. In the crystal structures of the cyclic tetradepsipeptides [26,27] conformer F is found. Of the three cyclic tetrapeptides containing proline,[6] c-L-Pro-Gly-L-Pro-Gly showed one major unsymmetric and one minor symmetric conformer and a low barrier to inversion (*ca.* 14 kcal/mol). The major conformer is suggested to be D again with the NH-amide bonds *trans*, and the minor species may adopt either conformer E or F. The c-L-Pro-Sar-L-Pro-Sar [6] was found to have the proline nitrogen as part of the *cis* amide bonds and showed no coalescence of the ring measured up to 70 °C and this is expected with N-CH_3 amide bonds *trans*.

The additional species observed in TFA solution for c-Ala-Sar_3, c-Gly-Sar_3, c-Gly-Sar-Gly-Sar and c-Gly_2-Sar_2 were in an earlier communication [12] attributed to protonation of one amide group. c-D-Ala-Sar-L-Ala-Sar, c-L-Ala_2-Sar_2 and c-Sar_4 which showed only one conformation in TFA were suggested to be rapidly protonated and deprotonated. No change in the NMR spectra were observed on changing the temperature, however, in c-Gly-Sar-Gly-Sar and c-Gly_2-Sar_2 the additional CH_2 lines coalesced at ca −15 °C. This indicates the presence of a second conformer stabilised in the polar solvent, and is now the preferred explanation of the observed spectra. The low observed coalescence temperature makes it unlikely that rotation of a *trans* amide group takes place while flipping of the *cis* amide groups is more reasonable. At temperatures below −15 °C the molecules adopt in addition to the c-Sar_4 conformation, conformers with one (Fig. 6 D) or both (Fig. 6 C) *cis* amide groups flipped. This also explains the additional conformer of c-Ala-Sar_3 and c-Gly-Sar_3 with one *cis* amide bond flipped (Fig. 6 D).

* R in Fig. 6 does not refer to a certain configuration of the amino acids but indicates the least hindered positions in the molecules.

** 1 cal = 4.184 J.

This conformer should have approximately the same NHCH- and CH_2-coupling constants as the c-Sar_4 conformer and corresponds to the observed NMR spectra. The only visible difference (Fig. 2) is that the CH_2 proton at lowest field in the major conformer has moved to higher field in the minor conformer and indicates that the adjacent *cis* amide bond has flipped. In c-D-Ala-Sar-L-Ala-Sar where no additional conformer is observed, flipping of the *cis* amide bonds is prohibited by the α-substituents.

For c-Gly_3-Sar and c-Gly_4 the conformational situation has not been finally elucidated. They may adopt, in solution, partly or entirely the c-Sar_4 conformation or one of the conformations discussed, but no indication is found for another amide sequence than alternating *cis* and *trans* amide bonds.

CYCLIC OCTAPEPTIDES

The cyclic octapeptides were studied by NMR spectroscopy, the method which gives the most informative picture of the conformational situation. By dissolving the peptides at low temperature (−50 °C), it should be possible to observe the conformation before any *cis,trans* isomerisation (needs *ca.* 20 kcal/mol) takes place. Slow warming of the sample in the NMR-tube and recording the NMR spectra should reveal any conformational changes.

c-(L-Ala-Sar_3)$_2$ (Fig. 7) when dissolved at −50 °C, shows one major and one minor (*ca.*

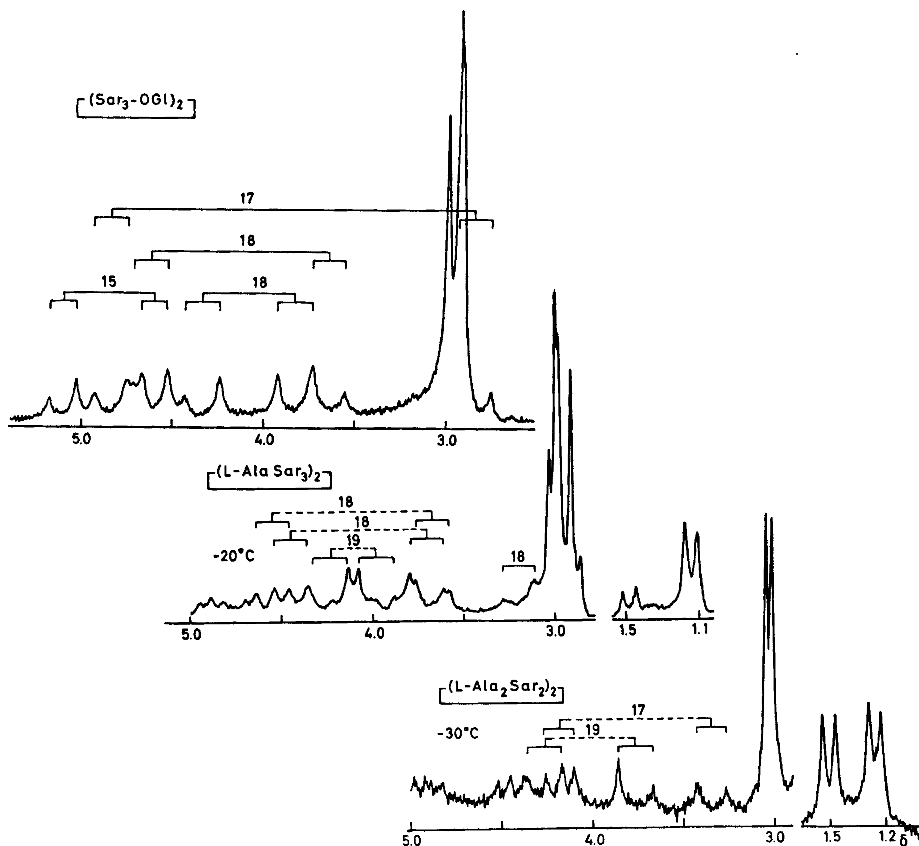


Fig. 7. The 100 MHz NMR spectrum of c-(L-Ala_2-Sar_2)$_2$ at −30 °C (lower spectrum) and c-(L-Ala-Sar_3)$_2$ at −20 °C (middle spectrum). Both were dissolved in $CHFCl_2$ with a trace of TFA at −50 °C and allowed to warm up in the probe (NH-region left out). The upper spectrum is of c-(Sar_3-OGl)$_2$ in $CDCl_3$ at −20 °C. OGl stands for $-OCH_2CO-$.

20 %) conformer both with one C-CH_3 doublet, three N-CH_3 singlets (1:1:1) and three CH_2 quartets (major). At about −15 °C the minor conformer starts to disappear, then the major conformer also disappears and new lines appear. At 5 °C the NMR spectrum consists of many N-CH_3 singlets and the CH_2 lines are broad and unresolved indicating that more than one conformer is present.

The NMR spectrum of the initially isolated c-(Gly-Sar_3)$_2$ shows at −50 °C only one conformer with two N-CH_3 lines (2:1) and with the CH_2 protons partially resolved. At about −20 °C the CH_2 protons start to coalesce and additional lines due to new conformers appear at −10 °C. Another sample which had been used for NMR measurements and subsequently isolated by evaporation of the solvent and treated with ether showed, however, at −50 °C a similar spectrum to that of c-(L-Ala-Sar_3)$_2$ with two conformers present.

c-(L-Ala_2-Sar_2)$_2$ (Fig. 7) is present in one conformer at −50 °C with two C-CH_3 doublets, two N-CH_3 singlets and two CH_2 quartets. At about −10 °C new conformers appear, and at 0 °C hardly any of the original lines can be seen and the lines in the CH_2 region are broad and unresolved.

c-(Gly_2-Sar_2)$_2$ shows at −70 °C two N-CH_3 singlets (1:1) and broad CH_2 lines suggesting that one conformer is present. The CH_2 protons started to coalesce already at about −50 °C, but the N-CH_3 protons did not coalesce until 0 °C. New conformers appeared at *ca.* −15 °C and hardly any of the initial conformer was present at 0 °C.

By comparing the NMR spectra of these four cyclic octapeptides, their conformations are shown to be closely related, all have centrosymmetry or two-fold axial symmetry and resemble the conformation of cyclooctasarcosyl [8,28] (Fig. in Ref. 8) and of the cyclic octadepsipeptide, c-(Sar_3-OGl)$_2$ [28] (Fig. 7) (OGl stands for the glycolic acid residue, $-OCH_2CO-$). The two latter take the same conformation both in the crystal and solution and undergo no conformational changes on changing the temperature. The X-ray structure of c-Sar_8 [29] showed approximately two-fold axial symmetry with the amide sequence *cis,cis,trans,trans,cis,cis, trans,trans*. The four cyclic octapeptides mentioned can easily adopt this skeleton by placing the *trans* preferred NH groups in the *trans* positions. For c-(L-Ala_2-Sar_2)$_2$ and c-(Gly_2-Sar_2)$_2$ only one possibility exists for arranging the amide groups on the c-Sar_8 skeleton, the one with the four *trans* amide positions occupied by the NH amides. In the case of c-(L-Ala-Sar_3)$_2$ and c-(Gly-Sar_3)$_2$ with two possibilities for the NH amide groups it is likely that the two conformations which are present in solution at low temperature differ only in having the NH amide groups in different *trans* positions. In c-Sar_4 and c-Sar_5 the most widely split CH_2-quartets belong to CH_2 groups adjacent to nitrogens which are part of *trans* amide bonds.[8] Applying this to the cyclic octapeptides and to the depsipeptide, the two most widely split CH_2-quartets in c-Sar_8 should belong to CH_2 groups adjacent to *trans* bonded nitrogen. In the depsipeptide one of these is shifted downfield and belongs to the CH_2 group adjacent to the ester oxygen which is part of a *trans* ester bond, while the other which corresponds to the most widely split quartet is present. In c-(L-Ala-Sar_3)$_2$ the most widely split quartet seems to be present in the minor conformation but is lacking in the major one, concluding that the NH group and the ester oxygen prefer different *trans* positions. Which one belongs to which cannot be decided.

Only c-Sar_8 and c-(Sar_3-OGl)$_2$ do not undergo any conformational changes while the cyclic octapeptides which contain NH amino acids are, on warming from −50 °C, transformed to more stable conformations in solution. This phenomenon was very marked in a series of cyclic pentapeptides [18] where several conformational changes took place, and the ability of the NH amides to form hydrogen bonds was proposed as the driving force.[24] Also here it is proposed that external hydrogen bonds stabilise the crystal conformers while internal hydrogen bonds stabilise the conformers in solution. The behaviour of the cyclic tetrapeptides which do not show the same phenomenon support this proposal as these rings are too small to form intramolecular hydrogen bonds.

c-Gly_8 which with its eight *trans*-preferred NH amide groups forms the other extreme of this series of cyclic octapeptides cannot adopt the c-Sar_8 skeleton. This molecule shows a simple NMR spectrum in TFA solution with one CH_2-doublet and one NH-triplet. No

coalescence phenomenon was observed on cooling the solution to −50 °C. (The solubility was to low when the peptide was added to cooled solvent). This shows that c-Gly_8 possesses a lower barrier to inversion than the corresponding sarcosine containing molecules.

EXPERIMENTAL

The general methods used for preparation of benzyloxycarbonyl-peptide methyl (*t*-butyl) esters, acids, 2,4,5-trichlorophenyl esters and of cyclic peptides are given in Ref. 8 (Z-Sar_2-OMe, Z-Sar_2-OH, Z-Sar_3-OTcp and c-Sar_3). Solvents used in the reactions were of analytical grade, the light petroleum had b.p. 40−60 °C. The monobedded ion-exchange resin, Amberlite, MB-1, analytical grade was used, and the eluent was methanol (water in the case of c-Gly_4). The fully protected linear peptides were prepared in methylene chloride, otherwise stated. After removal of the Z-group the residual peptide ester (confirmed by NMR) was 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 60A) and thin-layer chromatography performed on silica gel G in various solvent systems: 2 % methanol-chloroform (A), 5 % methanol-chloroform (B), 10 % methanol-chloroform (C), 15 % methanol-chloroform (D), 20 % methanol-chloroform (E), methanol-chloroform-water 70:20:10 (F), ethanol-water-acetic acid 85:10:5 (G).

Samples for elemental analysis of the Z-peptide esters were purified on a silica gel column, eluting with chloroform followed by chloroform added increasing amount of methanol 2−5 %. Abbreviations: Et_3N = triethylamine, DCC = dicyclohexylcarbodiimide, HOTcp = 2,4,5-trichlorophenol, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid, AcOH = glacial acetic acid.

For the NMR studies (Varian HA 100) where the peptides were dissolved at low temperature, the peptide was placed on a layer of glass wool in a glass tube inside the NMR-tube and held above the solvent by an O-ring. The whole arrangement was cooled to the desired temperature and the peptide pushed into the solvent by a glass rod which during the cooling was held inside the glass tube by another O-ring.

Cyclo-N-*methyl*-L-*alanyltrisarcosyl*. Z-L-MeAla-OH[30] (1.8 g = 7.6 mmol) and Sar_3-OMe[8] (1.8 g = 7.3 mmol) [obtained by hydrogenation of Z-Sar_3-OMe[8] (3.5 g = 9.2 mmol)] were dissolved in CH_2Cl_2 (50 ml) cooled to 0 °C and DCC (1.7 g = 8.2 mmol) added in portions. The reaction mixture was allowed to attain room temperature during 15 h and worked up as described.[8] Treatment with ether and light petroleum gave the Z-L-MeAla-Sar_3-OMe as a viscous oil (2.9 g = 86 %), homogeneous by TLC, R_F 0.45 (B). To Z-L-MeAla-Sar_3-OMe (2.5 g = 5.4 mmol) in methanol (20 ml) was added 1 N NaOH (5.6 ml), stirred at room temperature for 1.5 h and worked up as described.[8] The resulting Z-L-MeAla-Sar_3-OH (1.8 g) was purified by extraction from a CH_2Cl_2 solution into an alkaline layer, acidification with 2 N HCl and again extraction into CH_2Cl_2 or $CHCl_3$, followed by drying and evaporation gave a non crystalline viscous oil (1.6 g = 67 %), TLC, R_F 0.65 (G). Anal. $C_{21}H_{30}N_4O_7$: C, H. The acid (1 g = 2.2 mmol), HOTcp (0.6 g = 3 mmol) in CH_2Cl_2 (30 ml) and DCC (0.5 g = 2.4 mmol) added at 0 °C gave after being worked up as described,[8] the Z-L-MeAla-Sar_3-OTcp as a foamy solid (1.2 g = 86 %), TLC, R_F 0.45 (B). Anal. $C_{27}H_{31}N_4O_7Cl_3$: C, H. This (1 g = 1.6 mmol) was dissolved in methanol (50 ml) containing conc. HCl (0.1 ml) and 5 % Pd-C (0.2 g), hydrogenated for 50 min and worked up as described.[8] The resulting semi solid (0.7 g = 1.3 mmol = 83 %) of HCl. L-MeAla-Sar_3-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 2 h. After evaporation, the residue was taken into methanol (5 ml) and undissolved material filtered off (58 mg). This appeared to be pure cyclic tetrapeptide. The filtrate was diluted with methanol (60 ml) and passed through an ion-exchange column, eluting with methanol. The evaporated residue was taken into acetone (5 ml) and filtered. The precipitate contained cyclic tetrapeptide and some DC-urea which was removed by sublimation at reduced pressure. The filtrate was evaporated and the residue which contained more cyclic tetrapeptide, some DC-urea and traces of cyclic octapeptide (TLC) was purified on a small silica gel column, eluting with chloroform followed by chloroform added increasing amount of methanol (2−20 %) and sublimed. Yield of c-L-MeAla-Sar_3 (20 %), subl. temp. 180 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.5 (C), *m*/*e* 298, $[\alpha]_D^{22}$ + 16° (*c* 0.5, AcOH). Anal. $C_{13}H_{22}N_4O_4$: C, H, N.

Cyclo-L-*alanyltrisarcosyl*. Z-L-Ala-OH[31] (1.8 g = 8.1 mmol), Sar_3-OMe (1.9 g = 7.8 mmol) and DCC (1.8 g = 8.7 mmol) in CH_2Cl_2 (50 ml) gave the Z-L-Ala-Sar_3-OMe as a viscous foam (3.1 g = 88 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{21}H_{30}N_4O_7$: C, H, N. The ester (3 g = 6.7 mmol) in 1 N NaOH (7 ml) (1.5 h) resulted in the Z-L-Ala-Sar_3-OH (2.5 g = 86 %), softened at 80 °C, TLC, R_F 0.65 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, N. The acid (1.9 g = 4.3 mmol), HOTcp (1.1 g = 5.6 mmol) and DCC (0.9 g = 4.4 mmol) gave the Z-L-Ala-Sar_3-OTcp as a viscous foam (2.3 g = 88 %), TLC, R_F 0.7 (C), which (1.3 g = 2.1 mmol) was hydrogenated and the HCl.L-Ala-Sar_3-OTcp (1 g = 1.9 mmol = 90 %) cyclised and worked up as described earlier. The residue

after passage through an ion-exchange column was taken into methanol (5 ml) and undissolved DC-urea filtered off. The evaporated residue was taken into acetone (5 ml) and undissolved DC-urea and cyclic tetrapeptide filtered off, these were separated by sublimation. The filtrate was evaporated, treated with ether and sublimed, some more cyclic tetrapeptide and traces of cyclic octapeptide were isolated. Yields of cyclic compounds: c-L-Ala-Sar_3 (25 %), subl. temp. 190 °C/0.01 mmHg, m.p. 315 °C (subl.), TLC, R_F 0.45 (C), *m/e* 284, $[\alpha]_D^{22}+70.6°$ (*c* 0.5, AcOH). Anal. $C_{12}H_{20}N_4O_4$: C,H,N. c-Sar_2 (33 %), c-Ala-Sar (16 %) and c-(Ala-$Sar_3)_2$ (2 %).

Cyclo-L-alanyltrisarcosyl-L-alanyltrisarcosyl. Z-L-Ala-Sar_3-OMe (5.2 g = 11.5 mmol) in methanol (200 ml) was hydrogenated over Pd-C (1 g). The resulting oil (4.3 g = 10.4 mmol) of L-Ala-Sar_3-OMe, Z-L-Ala-Sar_3-OH (4.7 g = 10.8 mmol) and DCC (2.4 g = 11.6 mmol) added at −15 °C gave the Z-(L-Ala-$Sar_3)_2$-OMe (6.6 g = 86 %), softened at 85 °C, TLC, R_F 0.5 (D). This (4.2 g = 5.7 mmol) in NaOH (6 ml) (2 h) gave the Z-(L-Ala-$Sar_3)_2$-OH as a viscous foam (3.2 g = 78 %), TLC, R_F 0.8 (G) and the acid (3 g = 4.1 mmol), HOTcp (1.1 g = 5.5 mmol) and DCC (0.95 g = 4.6 mmol) resulted in the Z-(L-Ala-$Sar_3)_2$-OTcp (3 g = 83 %), softened at 65 °C, TLC, R_F 0.55 (D), which was hydrogenated and the HCl.(L-Ala-$Sar_3)_2$-OTcp (0.9 g = 1.1 mmol = 78 %) cyclised. The residue after passage through an ion-exchange column was taken into methanol (5 ml) and some DC-urea filtered off. The cyclic octapeptide crystallised out from acetone (5 ml) at 0 °C and traces of DC-urea was removed by sublimation (heated to 200 °C/0.01 mmHg). The remaining powder was pure c-(L-Ala-$Sar_3)_2$ (12 %), m.p. 345 °C (subl.) TLC, R_F 0.25 (F), *m/e* 568. Anal. $C_{24}H_{40}N_8O_8$: C, H, N.

Cyclo-D-alanylsarcosyl-L-alanylsarcosyl and cyclo-D-alanylsarcosyl-D-alanylsarcosyl (or cyclo-L-alanylsarcosyl-L-alanylsarcosyl).

From HCl.Sar-D-Ala-Sar-L-Ala-OTcp.

Z-Sar-OH [32] (23 g = 103 mmol), L-Ala-O^tBu [33] (15 g = 103 mmol) and DCC (23 g = 111 mmol) resulted in an oil (28 g = 78 %) of Z-Sar-L-Ala-O^tBu, TLC, R_F 0.6 (A). This (11 g = 31.4 mmol) was hydrogenated and the Sar-L-Ala-O^tBu (6.3 g = 29.1 mmol) together with Z-Sar-D-Ala-OH [18] (8.7 g = 29.6 mmol) and DCC (6.6 g = 32 mmol) gave the Z-Sar-D-Ala-Sar-L-Ala-O^tBu as a viscous foam (11 g = 77 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{24}H_{36}N_4O_7$: C, H, N. The ester (5 g = 10.1 mmol) was dissolved in TFA (30 ml) and kept at 20 °C for 1.5 h, evaporated and the residual oil added dropwise to ether (800 ml). The resulting solid was further purified, in the usual way, by extraction from a chloroform solution into an alkaline aqueous layer, acidification and extraction into chloroform and gave the Z-Sar-D-Ala-Sar-L-Ala-OH (3.8 g, 76 %) softened at 70 °C, TLC, R_F 0.7 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, N. This (3.5 g = 8 mmol), HOTcp (1.9 g = 9.6 mmol) and DCC (1.8 g = 8.7 mmol) resulted in Z-Sar-D-Ala-Sar-L-Ala-OTcp (4.4 g = 89 %) as a foamy solid, TLC, R_F 0.8 (C), which (3.5 g = 5.7 mmol) was hydrogenated and the HCl.Sar-D-Ala-Sar-L-Ala-OTcp (2.7 g = 5.2 mmol = 93 %) cyclised. After evaporation of the pyridine, the residue was taken into methanol (10 ml) and pure c-D-Ala-Sar-L-Ala-Sar (270 mg) filtered off. The filtrate was diluted with methanol (70 ml), passed through an ion-exchange column and the residue sublimed. Two cyclic tetrapeptides were isolated, at 220 and 290 °C (0.01 mmHg) with identical mass spectra and *m/e* 284 which is the required molecular weight for the cyclic tetrapeptide and its isomers. The IR spectra are different and the NMR spectra showed that the major product is c-D-Ala-Sar-L-Ala-Sar. The minor product is suggested to be c-D-Ala-Sar-D-Ala-Sar originated by racemisation. Yields of cyclic compounds: c-D-Ala-Sar-L-Ala-Sar (27 %), subl. temp. 290 °C/0.01 mmHg, m.p. > 350 °C. Anal. $C_{12}H_{20}N_4O_4$: C, H, N. c-D-Ala-Sar-D-Ala-Sar (1 %), subl. temp. 220 °C/0.01 mmHg, m.p. 340 °C (subl.), TLC, R_F 0.75 (E), $[\alpha]_D^{22}+92$ (*c* 0.3, AcOH).

From HCl.Sar-L-Ala-Sar-L-Ala-OTcp.

Z-Sar-L-Ala-O^tBu (12 g = 34 mmol) in TFA (50 ml) (1 h) gave the Z-Sar-L-Ala-OH (8 g = 80 %), m.p. 145 °C, TLC, R_F 0.75 (F). Anal. $C_{14}H_{18}N_2O_5$: C, H, which (5.9 g = 20 mmol), together with Sar-L-Ala-O^tBu (4.1 g = 19 mmol) and DCC (4.5 g = 21.8 mmol) gave the Z-Sar-L-Ala-Sar-L-Ala-O^tBu, a foamy solid (8.6 g = 92 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{24}H_{36}N_4O_7$: C, H, N. This (4.5 g = 9.1 mmol) in TFA (40 ml) (1 h) gave the Z-Sar-L-Ala-Sar-L-Ala-OH as a solid (3.1 g = 77 %), which (2.5 g = 5.7 mmol) together with HOTcp (1.3 g = 6.5 mmol) and DCC (1.3 g = 6.3 mmol) resulted in the Z-Sar-L-Ala-Sar-L-Ala-OTcp as a solid (2.9 g = 83 %), which (2.5 g = 4 mmol) was hydrogenated and the HCl.Sar-L-Ala-Sar-L-Ala-OTcp (1.8 g = 3.5 mmol = 86 %) cyclised. Only 8 mg of c-D-Ala-Sar-L-Ala-Sar precipitated from methanol (10 ml), the filtrate was diluted with methanol (50 ml) and passed through an ion-exchange column and the residue after evaporation sublimed. Only traces of c-L-Ala-Sar-L-Ala-Sar (or c-D-Ala-Sar-D-Ala-Sar) and about 10 mg (1 %) of c-D-Ala-Sar-L-Ala-Sar were isolated altogether.

From HCl.L-Ala-Sar-L-Ala-Sar-OTcp.

Z-Sar-L-Ala-OH (7 g = 23 mmol), HCl.Sar-OMe (3.2 g = 23 mmol), Et_3N (2.3 g = 23 mmol) and DCC (5.2 g = 25 mmol) resulted in Z-Sar-L-Ala-Sar-OMe, a viscous oil (7.5 g = 86 %), TLC, R_F 0.35 (A), R_F 0.65 (B). This (7 g = 18.5 mmol) was hydrogenated and the Sar-L-Ala-Sar-OMe (4.2 g = 17.1 mmol) together with Z-L-Ala-OH (4 g = 17.9 mmol) and DCC (3.9 g = 18.9 mmol) gave the Z-L-Ala-Sar-L-Ala-Sar-OMe as a solid (6.8 g = 88 %). The ester (6 g = 13.3 mmol) in NaOH (14 ml) (2 h) gave the Z-L-Ala-Sar-L-Ala-Sar-OH as a solid (5.1 g =

88 %), TLC, R_F 0.7 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, which (3.2 g = 7.3 mmol), HOTcp (1.7 g = 8.6 mmol) and DCC (1.6 g = 7.8 mmol), resulted in the Z-L-Ala-Sar-L-Ala-Sar-OTcp as a foam (4 g = 88 %), TLC, R_F 0.6 (C) and this (3 g = 4.9 mmol) was hydrogenated and the HCl.L-Ala-Sar-L-Ala-Sar-OTcp (2.1 g = 4 mmol = 84 %) cyclised. Only traces of c-D-Ala-Sar-L-Ala-Sar precipitated from methanol (70 ml), the filtrate was passed through an ion-exchange column and the residue after evaporation sublimed. The major product was c-Ala-Sar (64 %, calc. from two cyclisations per chain) and only traces (< 1 %) of c-D-Ala-Sar-L-Ala-Sar were isolated.

From HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp.

HCl.DL-Ala-OMe[34] (42 g = 302 mmol), Et_3N (31 g = 307 mmol), Z-Sar-OH[32] (69 g = 309 mmol) and DCC (68 g = 330 mmol) gave a viscous oil (84 g = 90 %) of Z-Sar-DL-Ala-OMe, TLC, R_F 0.65 (B). The ester (80 g = 259 mmol), in NaOH (262 ml) (2 h) resulted in Z-Sar-DL-Ala-OH (66 g = 85 %), TLC, R_F 0.75 (F), recrystallised from acetone, m.p. 120 °C. Anal. $C_{14}H_{18}N_2O_5$: C, H, N. The acid (45 g = 153 mmol), HCl.Sar-OMe[35] (21 g = 151 mmol), Et_3N (15.5 g = 153 mmol) and DCC (34 g = 165 mmol) resulted in Z-Sar-DL-Ala-Sar-OMe, a viscous oil (51 g = 88 %), TLC, R_F 0.4 (A), R_F 0.65 (B). Anal. $C_{18}H_{25}N_3O_6$: C, H. This (10 g = 26.3 mmol) was hydrogenated and the Sar-DL-Ala-Sar-OMe (6.1 g = 24.9 mmol), Z-DL-Ala-OH[31] (5.6 g = 25.1 mmol) and DCC (5.6 g = 27.2 mmol) resulted in the Z-DL-Ala-Sar-DL-Ala-Sar-OMe, a non crystalline solid (10 g = 89 %), TLC, R_F 0.45 (B). Anal. $C_{21}H_{30}N_4O_7$: C, H, N, which (9 g = 20 mmol) in NaOH (22 ml) (1.5 h) gave the Z-DL-Ala-Sar-DL-Ala-Sar-OH (7.7 g = 88 %), softened at 75 °C, TLC, R_F 0.7 (G). The acid (4 g = 9.1 mmol), HOTcp (2.1 g = 10.6 mmol) and DCC (2.1 g = 10.2 mmol) gave the Z-DL-Ala-Sar-DL-Ala-Sar-OTcp (5 g = 89 %), TLC, R_F 0.7 (C) which (3.5 g = 5.7 mmol) was hydrogenated and the HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp (2.6 g = 5 mmol = 89 %) cyclised. The precipitate from methanol (10 ml) was sublimed and consisted of c-D-Ala-Sar-L-Ala-Sar (60 mg) and c-D-Ala-Sar-D-Ala-Sar together with c-L-Ala-Sar-L-Ala-Sar (18 mg). The filtrate was concentrated on a rotary evaporator without heating and a second crop of the DD- and LL-isomers (31 mg) together with only traces of c-D-Ala-Sar-L-Ala-Sar precipitated. The solution was kept in a refrigerator overnight and some more of the DD and LL isomers plus traces of DC-urea were isolated. The filtrate was diluted with methanol (100 ml), passed through an ion-exchange column, evaporated and the residue treated with ether and sublimed and gave mostly c-Ala-Sar (74 mg). Yields of cyclic compounds: c-D-Ala-Sar-L-Ala-Sar (5 %), c-D-Ala-Sar-D-Ala-Sar together with c-L-Ala-Sar-L-Ala-Sar (4 %), $[\alpha]_D^{22} = 0$ (c 0.8, AcOH), c-Ala-Sar (52 %, calc. two cyclisations per chain).

Cyclo-di-L-alanyldisarcosyl.

From HCl.Sar-L-Ala_2-Sar-OTcp.

Z-L-Ala_2-OH[36] (15 g = 51 mmol) [prepared from Z-L-Ala-OH,[31] HCl.L-Ala-OMe[37] using DCC and the Z-L-Ala_2-OMe hydrolysed in NaOH], HCl.Sar-OMe (7.1 g = 51 mmol), Et_3N (5.2 g = 51 mmol) and DCC (11.5 g = 55 mmol) resulted in Z-L-Ala_2-Sar-OMe, a viscous oil (15.5 g = 80 %), TLC, R_F 0.4 (A), R_F 0.65 (B). This (20 g = 52.8 mmol) was hydrogenated and the L-Ala_2-Sar-OMe (12.2 g = 49.8 mmol) gave together with Z-Sar-OH (12 g = 53.8 mmol) and DCC (11.3 g = 54.8 mmol) the Z-Sar-L-Ala_2-Sar-OMe, a viscous oil (19.5 g = 87 %), TLC, R_F 0.4 (B). Anal. $C_{21}H_{30}N_4O_7$: C, H, N, which (11 g = 24 mmol) in NaOH (25 ml) (2.5 h) gave the Z-Sar-L-Ala_2-Sar-OH, a viscous oil (8.8 g = 83 %), TLC, R_F 0.7 (F). This (7 g = 16 mmol), HOTcp (3.7 g = 18.8 mmol) and DCC (3.7 g = 17.9 mmol) resulted in a solid (9.2 g = 93 %) of Z-Sar-L-Ala_2-Sar-OTcp, TLC R_F 0.7 (C), which (2.3 g = 3.7 mmol) was hydrogenated and the HCl.Sar-L-Ala_2-Sar-OTcp (1.8 g = 3.5 mmol = 94 %) cyclised. The residue after passage through an ion exchange column was taken into acetone (10 ml) and undissolved material filtered off. This was mostly DC-urea, some cyclic octapeptide and traces of the cyclic tetrapeptide (TLC, E) and could be separated by sublimation. The filtrate was evaporated and a new portion of acetone (5 ml) added, the precipitation was now pure cyclic tetrapeptide. The residue after evaporation consisted of cyclic tetrapeptide and some oily impurities. Sublimation sometimes led to decomposition of the cyclic tetrapeptide into cyclic dipeptides. A safer method for isolation was purification on a small silica gel column eluting with chloroform followed by chloroform containing methanol (2 – 5 %). Yields of cyclic compounds; c-L-Ala_2-Sar_2 (2.5 %), subl. temp. 240 °C/0.01 mmHg, m.p. 310 °C (subl.), TLC, R_F 0.2 (C), R_F 0.6 (E), *m/e* 284, $[\alpha]_D^{22} - 69°$ (c 0.5, AcOH), c-(L-Ala_2-Sar_2)$_2$ (3 %), subl. temp. 280 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.3 (F), R_F 0.15 (E), *m/e* 568.

From HCl.Sar_2-L-Ala_2-OTcp.

Z-Sar_2-L-Ala_2-O^{t}Bu[18] (6.9 g = 14 mmol) in TFA (40 ml) (1 h) gave the Z-Sar_2-L-Ala_2-OH as a solid (4.7 g = 77 %). Anal. $C_{20}H_{28}N_4O_7$: C, H. This (4.7 g = 10.8 mmol), HOTcp (2.6 g = 13.2 mmol) and DCC (2.5 g = 12 mmol) resulted in a solid (5.2 g = 78 %) of Z-Sar_2-L-Ala_2-OTcp, which (1.3 g = 2.1 mmol) was hydrogenated and the HCl·Sar_2-L-Ala_2-OTcp (1 g = 1.9 mmol = 91 %) cyclised. A white solid was left after passage through an ion-exchange column and evaporation, this was sublimed, and only c-Sar_2 (30 %) and c-Ala_2 (13 %) isolated.

Cyclo-tri-L-alanylsarcosyl. Z-L-Ala-OH (3 g = 13.4 mmol), L-Ala_2-Sar-OMe (3.2 g = 13 mmol) and DCC (3 g = 14.5 mmol) resulted in a solid (4.2 g = 71 %) of Z-L-Ala_3-Sar-OMe, TLC, R_F 0.6 (C) which (3 g = 6.6 mmol) in NaOH (7 ml) (2 h) gave the Z-L-Ala_3-Sar-OH as a

solid (2 g=69 %), TLC, R_F 0.65 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, N. The acid (1.6 g=3.6 mmol), HOTcp (0.9 g=4.5 mmol) and DCC (0.84 g=4 mmol) gave the Z-L-Ala_3-Sar-OTcp as a foamy solid (1.8 g=80 %), which (1.5 g=2.4 mmol) was hydrogenated and the HCl.L-Ala_3-Sar-OTcp (1 g=1.9 mmol=79 %) cyclised. The precipitate from methanol (10 ml) was sublimed and DC-urea and some (20 mg) cyclic tetrapeptide isolated. The filtrate was passed through an ion-exchange column and the evaporated residue which was small gave by sublimation a few mg more of cyclic tetrapeptide. Yield of c-Ala_3-Sar (4.5 %), subl. temp. 250 °C/0.01 mmHg, m.p. >350 °C, *m/e* 284. The NMR spectrum showed that the isolated cyclic tetrapeptide was either c-L-Ala_2-D-Ala-Sar or c-D-Ala_2-L-Ala-Sar and racemisation of one or two L-alanine residues had occurred.

Cyclo-tetra-L-alanyl.[2] Z-L-Ala_2-OH[36] (12 g=40.8 mmol), L-Ala_2-O^{t}Bu[18] (8.7 g=40.2 mmol) and DCC (9.1 g=44.1 mmol) gave the Z-L-Ala_4-O^{t}Bu (13.7 g=69 %), which (10 g=20 mmol) in TFA (40 ml) (1 h) gave the Z-L-Ala_4-OH[36] (7.6 g=85 %). The acid (3.9 g=8.9 mmol), HOTcp (2.1 g=10.6 mmol) and DCC (2 g=9.7 mmol) in DMF (20 ml) resulted in the Z-L-Ala_4-OTcp as a white powder (4.6 g=85 %), which (1.5 g=2.4 mmol) was hydrogenated and the HCl.L-Ala_4-OTcp (1.1 g=2.1 mmol=87 %) cyclised. The precipitate from methanol (25 ml) was sublimed, and appeared to be pure cyclic tetrapeptide (25 mg). The filtrate was passed through an ion-exchange column, the evaporated residue sublimed and some cyclic tetramer was isolated. Yield of c-Ala_4 (5 %), subl. temp. 200–240 °C/0.01 mmHg, m.p. 315 °C (subl.), *m/e* 284.

Cyclo-glycyltrisarcosyl. Z-Gly-OH[31] (7 g=33.4 mmol), Sar_3-OMe[6] (8 g=32.6 mmol) and DCC (7.5 g=36 mmol) resulted in a viscous foam (10.5 g=74 %) of Z-Gly-Sar_3-OMe, TLC, R_F 0.4 (B), R_F 0.6 (C). Anal. $C_{20}H_{28}N_4O_7$: C, H, N, which (9 g=20.6 mmol) in NaOH (22 ml) (2 h) gave the Z-Gly-Sar_3-OH (7.8 g=89 %), TLC, R_F 0.6 (G), m.p. 165 °C. The acid (4 g=9.4 mmol), HOTcp (2.2 g=11.1 mmol) and DCC (2.2 g=10.6 mmol) resulted in the Z-Gly-Sar_3-OTcp as a foamy solid (4.6 g=81 %), TLC, R_F 0.6 (C), which (2.4 g=4 mmol) was hydrogenated and the HCl.Gly-Sar_3-OTcp (1.8 g=35 mmol=90 %) cyclised. The precipitate from methanol (15 ml) was pure cyclic tetrapeptide (175 mg). The filtrate was passed through an ion-exchange column and the evaporated residue taken into methanol (10 ml). The precipitate consisted of cyclic tetrapeptide, DC-urea and some cyclic octapeptide (TLC, E,F) whereof the tetrapeptide and the DC-urea were isolated by sublimation (to 210 °C/0.01 mmHg) and the octapeptide remained as a white powder. The filtrate was after evaporation taken into acetone (10 ml) and DC-urea and cyclic octapeptide filtered off. The evaporated residue was treated with ether and sublimed. Small amounts of cyclic tetrapeptide and the two possible cyclic dipeptides were isolated. Yields of cyclic compounds: c-Gly-Sar_3 (23 %), subl. temp. 230 °C/0.01 mmHg, m.p. 322 °C, *m/e* 270, TLC, R_F 0.6 (E). Anal. $C_{11}H_{18}N_4O_4$: C, H, N. c-(Gly-Sar_3)$_2$ (3 %), subl. temp. 300 °C/0.01 mmHg (partly decomp.), m.p. 316 °C, *m/e* 540, TLC, R_F 0.3 (F), c-Gly-Sar (6 %), c-Sar_2 (4 %).

Cyclo-glycylsarcosylglycylsarcosyl. Z-Sar-OH (22.3 g=100 mmol), Gly-O^{t}Bu (13.1 g=100 mmol) and DCC (21 g=102 mmol) resulted in Z-Sar-Gly-O^{t}Bu (28.5 g=84 %), softened at 65 °C, TLC, R_F 0.5 (A). Anal. $C_{17}H_{24}N_2O_5$: C, H. This (20 g=59.5 mmol) in TFA (80 ml) (1 h) gave the Z-Sar-Gly-OH (14.4 g=86 %), m.p. 85 °C, TLC, R_F 0.7 (F), and the acid (13.5 g=48.2 mmol), HOTcp (12 g=61 mmol) and DCC (11 g=53.3 mmol) gave a foamy solid (19.6 g=89 %) of Z-Sar-Gly-OTcp which (10 g=22 mmol) together with Sar-Gly-O^{t}Bu (4.5 g=22 mmol) [obtained by hydrogenation of Z-Sar-Gly-O^{t}Bu (8 g=23.8 mmol)] were dissolved in CH_2Cl_2 (100 ml) and kept at 25 °C (4 days). The reaction mixture was diluted with $CHCl_3$, washed and worked up in the usual way to give the Z-Sar-Gly-Sar-Gly-O^{t}Bu (8.6 g=86 %), softened at 75 °C, TLC, R_F 0.55 (C). Anal. $C_{22}H_{32}N_4O_7$: C, H, N.

This (3 g=6.4 mmol) in TFA (40 ml) (1 h) gave the Z-Sar-Gly-Sar-Gly-OH (2.2 g=84 %), softened at 65 °C, TLC, R_F 0.7 (G), and the acid (2 g=4.9 mmol) and HOTcp (1.3 g=6.6 mmol) were dissolved in DMF (10 ml), diluted with CH_2Cl_2 (50 ml) and DCC (1.2 g=5.8 mmol) added at 0 °C. The mixture was allowed to attain room temperature during 15 h and the DC-urea filtered off together with some Z-Sar-Gly-Sar-Gly-OTcp which had precipitated out. The filtrate was worked up as usual and resulted in a solid (2 g=70 %) of Z-Sar-Gly-Sar-Gly-OTcp, TLC, R_F 0.65 (C), which (1.8 g=3 mmol) was hydrogenated and the HCl.Sar-Gly-Sar-Gly-OTcp (1.3 g=2.6 mmol=86 %) cyclised. The precipitate from methanol (20 ml) was cyclic tetrapeptide which crystallised from water as needles (280 mg). The methanol solution was passed through an ion-exchange column, but hardly any residue was left after evaporation. Yield of c-Gly-Sar-Gly-Sar (42 %), subl. temp. 260 °C/0.01 mmHg, m.p. >350 °C, *m/e* 256, TLC, R_F 0.5 (E). Anal. $C_{10}H_{16}N_4O_4$: C, H, N.

Cyclo-diglycyldisarcosyl

From HCl·Sar-Gly_2-Sar-OTcp.

Z-Gly_2-OH[38] (30 g=112 mmol) [prepared from H-Gly_2-OH, Z-Cl and NaOH in the usual way, recrystallised from water], HOTcp (28 g=142 mmol) and DCC (26 g=126 mmol) were dissolved in DMF (150 ml) and stirred at 25 °C for 24 h. The precipitated DC-urea was filtered off and the evaporated residue washed with ether to give a white solid (47 g=94 %) of Z-Gly_2-OTcp. This (45 g=101 mmol) together

with HCl.Sar-OMe (14 g = 100 mmol) and Et_3N (10.5 g = 103 mmol) were suspended in DMF (20 ml) and CH_2Cl_2 (100 ml), kept at 25 °C for 3 days and the now clear solution evaporated. The residue which was dissolved in $CHCl_3$ (400 ml) and washed as usual gave the Z-Gly_2-Sar-OMe (31 g = 88 %), TLC, R_F 0.45 (B). An analytical sample melted at 92 °C. Anal. $C_{16}H_{21}N_3O_6$: C, H, N. The ester (27 g = 76.9 mmol) was hydrogenated and the Gly_2-Sar-OMe (15.2 g = 70 mmol) together with Z-Sar-OH (16 g = 71.7 mmol) dissolved in CH_2Cl_2 (500 ml) and DCC (16 g = 77 mmol) added at − 10 °C. When the reaction mixture attained room temperature (15 h), the precipitate was filtered off. This consisted of DC-urea and some tetrapeptide ester which could be isolated by suspending the precipitate in $CHCl_3$ and filtration of insoluble DC-urea. The CH_2Cl_2-solution was diluted with $CHCl_3$ (500 ml) and worked up in the usual way to give the Z-Sar-Gly_2-Sar-OMe (25.8 g = 87 %). A sample (0.3 g) was further purified. TLC, R_F 0.5 (C), m.p. 162 °C. Anal. $C_{19}H_{26}N_4O_7$: C, H, N.

The ester (10 g = 23.7 mmol) in NaOH (25 ml) (1 h) gave the Z-Sar-Gly_2-Sar-OH (7.3 g = 75 %), TLC, R_F 0.7 (F). A sample was recrystallised from methanol with added traces of water, m.p. 147 °C. Anal. $C_{18}H_{24}N_4O_7$: C, H, N, and the acid (4.5 g = 11 mmol), HOTcp (2.6 g = 13.2 mmol) and DCC (2.5 g = 12.1 mmol) resulted in the Z-Sar-Gly_2-Sar-OTcp (5 g = 77 %), TLC, R_F 0.55 (C), m.p. 165 °C, which (2 g = 3.4 mmol) was hydrogenated and the HCl.Sar-Gly_2-Sar-OTcp (1.3 g = 2.6 mmol = 77 %) cyclised. After evaporation of the pyridine, the residue was dissolved in methanol (10 ml) and passed through a small silica gel column eluting with methanol. A yellow oil was first eluted, then a white powder which appeared to be the cyclic octapeptide. The oil was dissolved in methanol (80 ml), passed through an ion-exchange column and the evaporated residue taken into methanol (5 ml). The undissolved material (15 mg) appeared to be cyclic tetrapeptide and some more could be isolated when the filtrate was evaporated, treated with ether and sublimed. If the residue is oily, sublimation of it tends to decompose the cyclic tetrapeptide into cyclic dipeptides, and it is recommended to purify on a small silica gel column, eluting with chloroform, followed by chloroform containing methanol (2 − 5 %). Yields of cyclic compounds: c-Gly_2-Sar_2 (3 %), subl.temp. 260 °C/0.01 mmHg, m.p. 310 °C, TLC, R_F 0.4 (E), *m/e* 256. c-(Gly_2-Sar_2)$_2$ (13 %), m.p. > 350 °C, TLC, R_F 0.3 (F). Anal. $C_{20}H_{32}N_8O_8$: C, H, N.

From HCl.Sar_2-Gly_2-OTcp.

Z-Sar_2-OTcp (9 g = 19 mmol) [prepared from Z-Sar_2-OH, HOTcp and DCC] and H-Gly_2-O^{t}Bu (3.6 g = 19.1 mmol) [obtained by hydrogenation of Z-Gly_2-O^{t}Bu [39] (6.5 g = 20.1 mmol), prepared from Z-Gly-OH, H-Gly-O^{t}Bu using DCC] were dissolved in CH_2Cl_2 (150 ml), kept for 3 days at 25 °C and worked up in the usual way to give the Z-Sar_2-Gly_2-O^{t}Bu (7.8 g = 88 %), TLC, R_F 0.5 (C). An analytical sample melted at 141 °C. Anal. $C_{22}H_{32}N_4O_7$: C, H, N.

The ester (4 g = 8.6 mmol) in TFA (25 ml) (1 h) gave the Z-Sar_2-Gly_2-OH which was suspended in acetone and filtered (3 g = 85 %), m.p. 135 °C, TLC, R_F 0.7 (G). The acid (2.7 g = 6.6 mmol), HOTcp (1.6 g = 8.1 mmol) and DCC (1.5 g = 7.3 mmol) gave the Z-Sar_2-Gly_2-OTcp as a foamy solid (3.2 g = 84 %), TLC, R_F 0.75 (C), which (1.5 g = 2.5 mmol) was hydrogenated and the HCl.Sar_2-Gly_2-OTcp (1 g = 2 mmol = 83 %) cyclised. After passage through an ion-exchange column, the evaporated residue was taken into methanol (10 ml) and undissolved material which consisted of cyclic octapeptide (15 mg) and traces of cyclic tetrapeptide (TLC) filtered off. The evaporated filtrate was treated with ether and sublimed and gave mainly cyclic dipeptides. Yields of cyclic compounds: c-Gly_2-Sar_2 (traces), c-(Gly_2-Sar_2)$_2$ (3 %), c-Sar_2 (25 %), c-Gly_2 (10 %).

From HCl.Gly-Sar_2-Gly-OTcp.

Z-Sar-OH (3.5 g = 15.7 mmol), Sar-Gly-O^{t}Bu (3.2 g = 15.8 mmol) and DCC (3.7 g = 17.9 mmol) gave the Z-Sar_2-Gly-O^{t}Bu as a viscous oil (5.5 g = 13.5 mmol = 86 %), TLC, R_F 0.4 (A), R_F 0.6 (B). This was hydrogenated and the Sar_2-Gly-O^{t}Bu (3.4 g = 12.4 mmol) together with Z-Gly-OH (2.7 g = 12.9 mmol) and DCC (2.8 g = 13.6 mmol) resulted in the Z-Gly-Sar_2-Gly-O^{t}Bu as a foamy solid (4.8 g = 84 %), which (4 g = 8.6 mmol) in TFA (50 ml) (1.2 h) gave the Z-Gly-Sar_2-Gly-OH (2.9 g = 83 %). This (2.5 g = 6.1 mmol), HOTcp (1.5 g = 7.6 mmol) and DCC (1.4 g = 6.8 mmol) in DMF (20 ml) and CH_2Cl_2 (100 ml) gave the Z-Gly-Sar_2-Gly-OTcp as a solid (3.1 g = 87 %), which (1.3 g = 2.2 mmol) was hydrogenated and the HCl·Gly-Sar_2-Gly-OTcp (1 g = 2 mmol = 92 %) cyclised. The residue after passage through an ion-exchange column consisted of pure c-Gly-Sar (TLC) and sublimation gave 57 % (calc. two cyclisations per chain). No cyclic tetrapeptide nor cyclic octapeptide were observed.

Cyclo-triglycylsarcosyl. Z-Sar-Gly-OTcp (8.4 g = 18.3 mmol) [prepared from Z-Sar-Gly-OH, HOTcp and DCC] and Gly_2-O^{t}Bu (3.5 g = 18.6 mmol) in CH_2Cl_2 (100 ml) at 25 °C (3 days) resulted in Z-Sar-Gly_3-O^{t}Bu (7.3 g = 87 %), TLC, R_F 0.7 (E). An analytical sample melted at 155 °C. Anal. $C_{21}H_{30}N_4O_7$: C, H, N. This (4.5 g = 10 mmol) in TFA (25 ml) (1 h) gave the Z-Sar-Gly_3-OH which was suspended in acetone and filtered (3.2 g = 82 %), m.p. 197 °C, TLC, R_F 0.75 (G) and the acid (2.5 g = 6.3 mmol), HOTcp (1.6 g = 8.1 mmol) and DCC (1.5 g = 7.2 mmol) in DMF (25 ml) gave the Z-Sar-Gly_3-OTcp as a white powder which was suspended in acetone and filtered (2.4 g = 66 %) and (1.8 g = 3.1 mmol) hydrogenated and the HCl.Sar-Gly_3-OTcp (1.3 g = 2.7 mmol = 87 %) cyclised. After evaporation of the pyridine,

the residue was dissolved in methanol-water 1:1 (80 ml) and the precipitated DC-urea filtered off. The filtrate was passed through an ion-exchange column, eluting with methanol-water 1:1 and the evaporated residue dissolved in a small amount of water from which c-Gly_3-Sar crystallised as needles (3 %), subl.temp. 270 °C/0.01 mmHg, m.p. > 350 °C, *m/e* 242, TLC, R_F 0.3 (E), R_F 0.7 (F).

Cyclo-tetraglycyl.[1] Z-Gly_4-O^tBu was prepared from Z-Gly_2-OTcp and H-Gly_2-O^tBu in pyridine and hydrolysed in TFA to Z-Gly_4-OH which together with ethyl chloroformate and thioglycollic acid followed by treatment with HBr in acetic acid gave the HBr.Gly_4-SCH_2COOH.[1] This (0.5 g = 1.2 mmol) in DMF (100 ml) was added to stirred pyridine (600 ml) at 115 °C over a period of 4 h and stirred for another 4 h. Some precipitated polymer was filtered off and the evaporated residue dissolved in water (50 ml), passed through an ion-exchange column, eluting with water and by dissolving the evaporated residue in water (5 ml) a "white powder" which appeared to be c-Gly_4 (12 mg, 4.4 %) crystallised out, subl.temp. 270 °C/0.01 mmHg, m.p. 340 °C (subl.), *m/e* 228. The filtrate was evaporated to *ca.* 1 ml and ethanol (*ca.* 2 ml) added, small needles of c-Gly_8 (8 mg, 3 %) crystallised out, m.p. > 350 °C, *m/e* 456.

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