Lanthionine Chemistry. Part 5.[1] Synthesis of Cyclic Non-symmetrical Lanthionyl Peptides

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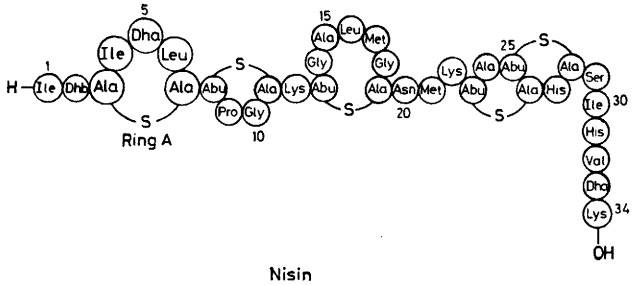
The synthesis of a protected fragment of the antibiotic nisin, specifically N^{α}-benzyloxycarbonyl-D-hemilanthionyl-L-isoleucyldehydroalanyl-L-leucyl-L-hemilanthionine α'-methyl ester (B) and of its analogue N^{α}-benzyloxycarbonyl-L-hemilanthionyl-L-isoleucyl-L-seryl-L-leucyl-L-hemilanthionine α'-methyl ester (A) is described. For the synthesis of these unusual peptidic compounds, unsymmetrically substituted derivatives of L- or *meso*-lanthionine were used. For the synthesis of compound (B), the *O*-diphenylphosphorylserine portion of the peptide (26) was transformed by a β-elimination reaction to the dehydroalanine group in peptide (27), which after partial deprotection was subjected to cyclization. Compound (A) is a precursor of the dehydroalanine-containing *cyclo*-L-lanthionyl peptide. The monomeric structures of the *cyclo*-peptides (A) and (B) were proved by molecular-weight determination and the presence of the amino–acrylic acid grouping in the molecule of (B) was ascertained by amino-acid analysis and its i.r. spectrum.

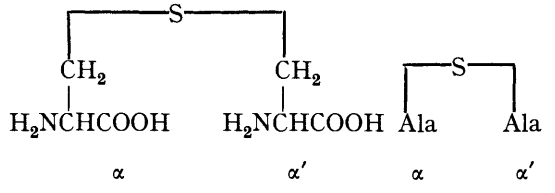
ELUCIDATION of the structure of the antibiotics nisin and subtilin by Gross *et al.*[2a] revealed that they are built of non-symmetrical cyclic heterodetic lanthionyl or β-methyl-lanthionyl peptides of different size and of linear peptide fragments, all interconnected through peptide bonds. Another unusual feature of these peptide antibiotics is the incorporation of unsaturated α-amino-acids into their peptide chain. We now present a synthetic



approach to these structures and deal specifically with the synthesis of two non-symmetrical amino- and carboxy-protected cyclopeptides (A) and (B) † containing L- or *meso*-lanthionine respectively. Compound (B) corresponds to the first of the nisin rings.

RESULTS AND DISCUSSION

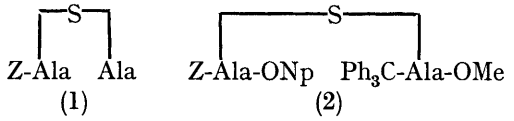
The main difficulties encountered in this work were related to (*i*) the incorporation into the peptide chain of αβ-unsaturated α-amino-acids which in the free form are



Lanthionine

unstable, being immediately converted to ammonia and α-keto-acids, and (*ii*) the chemical differentiation of the two amino- (α,α') and the two carboxy- (α,α') groups of lanthionine.

The first difficulty can be overcome by incorporation of a β-hydroxy-amino-acid group into the peptide chain as a precursor for the α-amino-acrylic acid residue. In fact serine derivatives bearing a good leaving group (toluene-*p*-sulphonyl,[3] diphenylphosphoryl,[3,4] or even acyl [5] groups) can be easily transformed to α-amino-acrylic acid residues by β-elimination. The second problem, *i.e.* differentiation of the functional lanthionine groups, can be solved as has been shown previously [1] by using as starting materials N^{α}-benzyloxycarbonyl-L-lanthionine (1a), N^{α}-benzyloxycarbonyl-(D ⟶ L)-lanthionine (1b),‡ and N^{α}-benzyloxycarbonyl-(L ⟶ D)-



Z = $PhCH_2OCO$

a; α,α' = L,L
b; α,α' = D,L
c; α,α' = L,D

lanthionine (1c), and converting these compounds through a series of reactions to compounds (2).

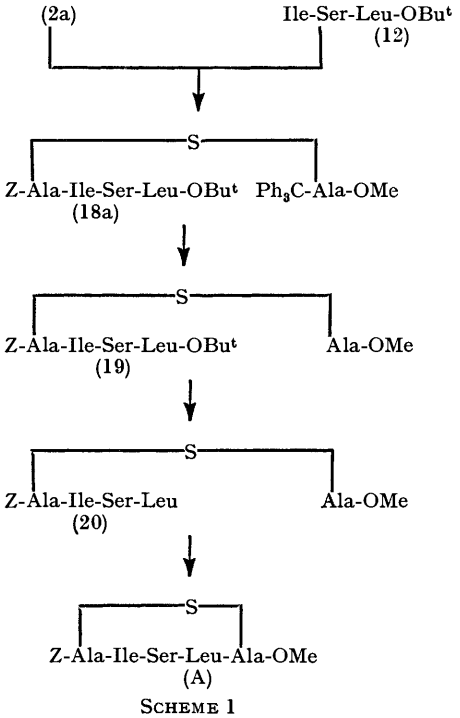
The active L-lanthionine ester (2a) thus formed, has now been coupled (Scheme 1) with the tripeptide L-isoleucyl-L-seryl-L-leucine t-butyl ester (12) to yield the peptide (18a). After partial deprotection of (18a) by treatment first with hydrogen chloride in methanol [6] and then with trifluoroacetic acid, cyclization of the resulting peptide (20) by means of hydroxysuccinimide-assisted dicyclohexylcarbodi-imide [7] led to the preparation of the *N*-protected cyclic peptide (A). Attempts to convert the free hydroxy-group of the serine residue into an ester group (*i.e.* toluene-*p*-sulphonyl or diphenylphosphoryl ester group) failed, presumably because of steric hindrance. Steric hindrance, caused probably by the bulky diphenylphosphoryl group, also prevented the cyclization of compound (24), prepared

† Another approach towards the synthesis of compound (B) has been announced by Gross *et al.*[2b]

‡ For the nomenclature of *meso*-lanthionine derivatives *cf.* Part 4.[1]

(Scheme 2) by condensation of (2b) with the tripeptide L-isoleucyl-*O*-diphenylphosphoryl-L-seryl-L-leucine (13), followed by detritylation with hydrochloric acid in acetic acid.

In view of these observations, we applied the series of

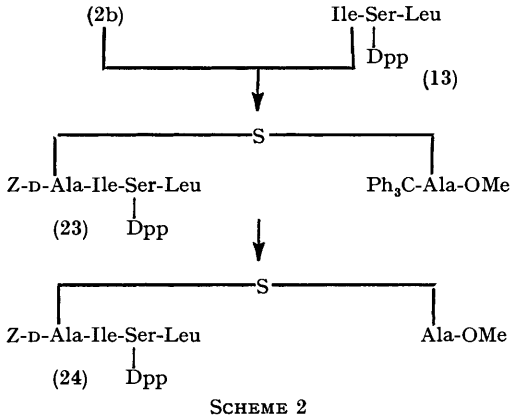


SCHEME 1

reactions shown in Scheme 3 for the synthesis of the cyclopeptide (B). The serine portion of compound (25) was thus transformed to its *O*-diphenylphosphoryl derivative (26). β-Elimination, caused by the action of sodium methylate, followed by selective saponification of the leucine methyl ester group,* afforded the dehydroalanyl peptide (28). Detritylation followed by cyclization with dicyclohexylcarbodi-imide in the presence of 1-hydroxy-benzotriazole [8] led to the formation of substance (B). Purification of this final cyclic peptide was effected by gel filtration on Sephadex LH 20.

* The other methyl ester group is virtually unaffected by alkali at room temperature, being sterically protected by the trityl group.[1,6]

The monomeric structure of both *cyclo*-lanthionyl derivatives (A) and (B) was proved by molecular-weight determinations. In addition to elemental analysis, the presence of dehydroalanine in peptide (B) was ascertained by the absence of serine and the presence of ammonia in the Moore and Stein amino-acid analysis of an acid hydrolysate, and by the demonstration of i.r. absorption bands characteristic of the aliphatic methylene double bond. The retention of the L-configuration of the leucine group during the cyclization step was proved for compound (B), by digestion of an acid hydrolysate with L-amino-acid oxidase, using norleucine as internal

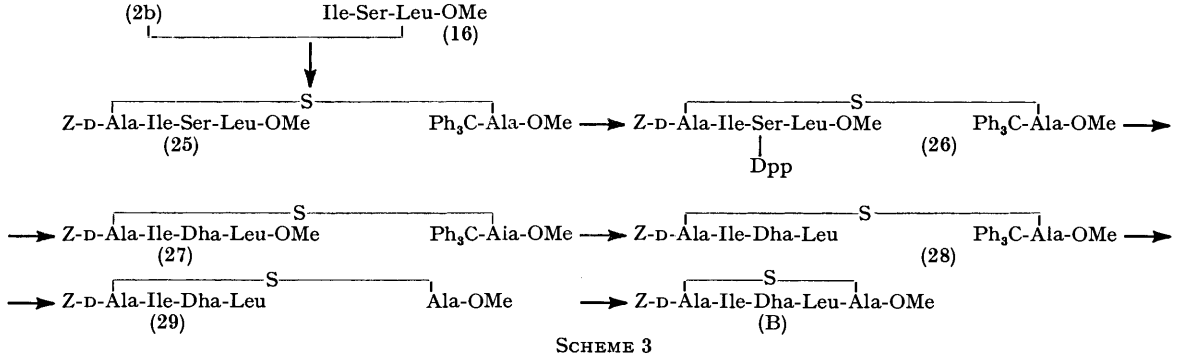


SCHEME 2

standard. After 20 h incubation (in tris–HCl buffer pH 7.2; 0.01M; 22 °C) the quantitative amino-acid analysis showed a remainder of: norleucine 1.09%; isoleucine 0.75%; and leucine 3.32%.

EXPERIMENTAL

The general instructions given in Part 4 [1] apply. The molecular-weight determination of compound (A) was performed at the Analytical Laboratory of A. Bernhardt, Elbach, Germany. Amino-acid analyses of peptide hydrolysates, the molecular-weight determination of compound (B), and the digestion of its acid hydrolysate with L-amino-acid oxidase, were performed at the Analytical Laboratory of F. Hoffmann–La Roche, Basle, Switzerland. The amino-acid analyses were carried out, using a Beckman amino-acid analyser (Typ Unichrom) according to the method of Spackman *et al.*[9] Additional solvent systems for t.l.c. were (8) butan-1-ol–ethanol–water (5 : 1 : 4, upper phase);



SCHEME 3

(9) acetonitrile–water (3 : 1); (10) ethyl acetate–methanol (7 : 3); (11) chloroform–methanol (4 : 1).

General Synthetic Procedures.—(a) *Peptide couplings.* (i) *By the DCC method.* This was performed by using dicyclohexylcarbodi-imide and *N*-hydroxysuccinimide at −10 °C in amounts 5% in excess of that of the carboxy-component. The reaction normally proceeded for 5 h at room temperature, whereupon the dicyclohexylurea was filtered off, the filtrate evaporated, and the residue dissolved in ethyl acetate and washed as described in (b).

(ii) *By the mixed anhydride method.* The mixed anhydride of the *N*-protected amino-acid (7.5 mmol) prepared in tetrahydrofuran (15 ml) by addition of isobutyl chloroformate (0.96 ml) and *N*-methylmorpholine (0.91 ml) (stirring for 10 min at −15 °C) was added to the free amino-component dissolved in tetrahydrofuran (15 ml) and the mixture stirred for 30 min at 0 °C, and then for 1 h at room temperature; it was evaporated, the residue dissolved in dimethylformamide (10 ml), the stirred solution treated at 0 °C with aqueous 2M-potassium hydrogencarbonate until pH 7.5 (moist indicator paper), and the mixture extracted with ethyl acetate and washed as described in (b).

(b) *Isolation of coupled product.* The mixture was washed successively with water, aqueous 10% citric acid, water, 2M-potassium hydrogencarbonate, and water to neutrality, then dried and evaporated.

Deprotection of Peptides and Isolation of the Corresponding Salts.—(a) *Removal of the benzyloxycarbonyl group by hydrogenolysis.* The *N*-protected peptide (2 mmol) in methanol (15 ml) containing 1% acetic acid was hydrogenolysed over palladium–charcoal (10%; 100 mg) for 2.5 h. The catalyst was filtered off, the filtrate evaporated, and the residue dissolved in 0.4N-hydrogen chloride in methanol (1.5 mol. equiv.) at −15 °C. The hydrochloride was precipitated with ether and recrystallized as indicated.

(b) *Removal of the benzyloxycarbonyl and the t-butyl ester groups by hydrogen bromide in acetic acid.* The *NO*-protected peptide (2 mmol) was treated with hydrogen bromide in acetic acid (2N; 7 ml) at room temperature. After 45 min, ether was added to precipitate the hydrobromide.

(c) *Removal of the* o-*nitrophenylsulphenyl group.* To a solution (or suspension) of the *N*-protected peptide (10 mmol) in the indicated solvent (20 ml) were added 2-mercaptoethanol (1.25 ml) and hydrogen chloride in ether (7N; 3.5 ml). After a few min the hydrochloride was precipitated with ether.

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*hemilanthionyl*-L-*isoleucyl*-L-*seryl*-L-*leucine t-Butyl Ester* N$^{\alpha'}$-*Trityl*-L-*hemilanthionine* α'-*Methyl Ester* (18a).—A solution of L-isoleucyl-L-seryl-L-leucine t-butyl ester hydrochloride (12) (3.85 g, 9 mmol) in dimethylformamide (15 ml) was cooled to 0 °C. Triethylamine (1.28 ml) and N$^{\alpha}$-benzyloxycarbonyl-N$^{\alpha'}$-trityl-L-lanthionine α-*p*-nitrophenyl α'-methyl diester (2a) (8.64 g, 12 mmol) dissolved in dimethylformamide (20 ml) were added and the solution was stored at room temperature for 12 h. Water was added and the mixture was extracted twice with ethyl acetate. The organic layer was washed successively with 2N-ammonia, water, aqueous 10% citric acid, and water, then dried and evaporated. The residue (7.9 g) was dissolved in chloroform, triethylamine (4 ml) was added, and the solution was stirred for 1 h. It was then washed as before, dried, and evaporated. The product was recrystallised from ethyl acetate–light petroleum to give the *protected tetrapeptide.* Yield and physical data are given in Table 1.

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*hemilanthionyl*-L-*isoleucyl*-L-*seryl*-L-*leucine t-Butyl Ester* L-*Hemilanthionine* α'-*Methyl Ester Hydrochloride* (19).—The trityl peptide (18a) (1.85 g, 1.9 mmol) was treated with hydrogen chloride in methanol (1N; 1.9 ml) for 30 min at room temperature. The solution was evaporated and the residue was recrystallised from chloroform–light petroleum, giving the *hydrochloride.* Yield and physical data are given in Table 2.

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*hemilanthionyl*-L-*isoleucyl*-L-*seryl*-L-*leucine* L-*Hemilanthionine* α'-*Methyl Ester* (20).—The partially protected peptide (19) (3.5 g, 4.5 mmol) was treated with trifluoroacetic acid (45 ml; 95%, 40 min) at room temperature. Ethyl acetate was added and the solution was evaporated. Addition of ethyl acetate and evaporation were repeated 5—6 times to remove the excess of trifluoroacetic acid. The residue was crystallized from ethyl acetate–light petroleum and was dissolved in hot water. Addition of pyridine (pH 7) precipitated the *tetrapeptide.* Yield and some physical data are given in Table 2; on paper electrophoresis (250 V; 2.5 h) in the system 1N-hydrochloric acid (50 ml)–1M-tris(hydroxymethyl)aminomethane (114 ml)–water (to 1 l) (pH 9), the compound moved as a single band towards the anode.

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*hemilanthionyl*-L-*isoleucyl*-L-*seryl*-L-*leucyl*-L-*hemilanthionine* α'-*Methyl Ester* (*A*).—Compound (20) (3 g, 4.5 mmol) was dissolved in dimethylformamide (280 ml), the solution was cooled to −20 °C, and *N*-hydroxysuccinimide (2.1 g) and dicyclohexylcarbodi-imide (0.93 g), dissolved in a small volume of dimethylformamide, were added. To the solution, stirred for 2 h, dicyclohexylcarbodi-imide (0.93 g in dimethylformamide) was added again and the mixture stored at 4 °C. After 24 and 48 h two more portions of dicyclohexylcarbodi-imide (0.93 g each) were added and the mixture was then stored in the dark at room temperature. After 4 d, the solution gave no colour with ninhydrin. Acetic acid (4 ml; 50%) was added and after 5 min the mixture was evaporated under high vacuum. The residue was triturated with tetrahydrofuran and was filtered off. Trituration of the undissolved material with dimethyl sulphoxide was followed by filtration to remove dicyclohexylurea and addition of water to precipitate the *cyclic peptide* (1 g), m.p. 262—265 °C (decomp.); this peptide was recrystallized from the same solvent mixture (0.9 g, 35%), m.p. 264—267 °C (decomp.), $[\alpha]_D^{30}$ −78.5° (*c* 2 in dimethyl sulphoxide, R_F (9) 0.65 (Found: C, 55.2; H, 6.9; N, 10.7; O, 22.2; S, 4.9. $C_{30}H_{45}N_5O_9S$ requires C, 55.3; H, 7.0; N, 10.7; O, 22.1; S, 4.9%); amino-acid analysis: Ser, 0.8; Ile, 1.0; Leu, 1.0.* Lanthionine was detected qualitatively.[10] Molecular weight determination (osmometrically in dimethylformamide solution, in three concentrations): 642, 649, 645 (calc. 651.8).

L-*Hemilanthionyl*-L-*isoleucyl*-L-*seryl*-L-*leucyl*-L-*hemilanthionine* α'-*Methyl Ester Picrate* (30).—The *N*-protected compound (A) (0.6 g, 0.92 mmol) was dissolved in trifluoroacetic acid (45 ml), phosphorous acid diethyl ester (12 ml), and methyl ethyl sulphide (12 ml). Hydrogen bromide was bubbled in the dark through the solution for 1 h at −5 °C and the mixture was set aside in a tightly closed container for 2 h at room temperature and then evaporated. The hygroscopic hydrobromide was precipitated with

* This amino-acid analysis was performed at the Biochemistry Department, Mount Sinai School of Medicine, New York, using a Beckman-Spinco amino-acid analyser (model 120C).

ether, dissolved in hot water, and heated with a saturated solution of sodium picrate. The *picrate* was recrystallized from methanol. Yield and physical data are given in Table 2.

N$^{\alpha}$-Benzyloxycarbonyl-D-hemilanthionyl-L-isoleucyl-O-diphenylphosphoryl-L-seryl-L-leucine Methyl Ester N$^{\alpha'}$-Trityl-L-hemilanthionine α'-Methyl Ester (26).—Diphenylphosphoryl chloride (160 mg, 0.59 mmol) was added to a solution of compound (25) (262 mg, 0.282 mmol) in pyridine (0.6 ml) at 0 °C. After 1 h at room temperature, aqueous 2% citric acid was added to precipitate an oily product which was solidified by decanting the supernatant liquid, adding water and scratching. It was collected by filtration and dissolved in ethyl acetate (30 ml). The solution was dried and evaporated to a small volume. The *peptide* was precipitated with light petroleum. Yield and physical data are given in Table 2.

N$^{\alpha}$-Benzyloxycarbonyl-D-hemilanthionyl-L-isoleucyl-dehydroalanyl-L-leucine Methyl Ester N$^{\alpha'}$-Trityl-L-hemilanthionine α'-Methyl Ester (27).—The *O*-protected peptide (26) (197 mg, 0.17 mmol) was dissolved in methanol (2.8 ml) with gentle heating; the solution was allowed to cool and 1N-sodium methylate in methanol (0.85 ml) was added. After 10 min the solution was acidified with aqueous 10% citric acid and diluted with water. The precipitated solid was filtered off and dissolved in ethyl acetate. The extract was washed successively with water, aqueous 2M-sodium hydrogencarbonate, and water, then dried and evaporated to a small volume. Addition of light petroleum precipitated out the *product*. Yield and physical data are given in Table 2.

N$^{\alpha}$-Benzyloxycarbonyl-D-hemilanthionyl-L-isoleucyl-dehydroalanyl-L-leucine N$^{\alpha'}$-Trityl-L-hemilanthionine α'-Methyl Ester (28).—The protected peptide diester (27) (2.3 g, 2.5 mmol) was dissolved in acetone (10 ml) and treated with stirring with 1N-sodium hydroxide (2.75 ml) added during 10 min. After 1 h the solution was diluted with water (100 ml), some undissolved material was filtered off, and the filtrate was acidified with aqueous 10% citric acid and stored at 4 °C overnight. It was then extracted twice with ethyl acetate and the extract was washed with water, dried, and evaporated to a small volume. Addition of light

TABLE 1

Protected peptides *

(3) Nps-Ser-Leu-OBu^{t}
(7) Z-Ile-Ser-Leu-OBu^{t}
(8) Z-Ile-Ala(3Cl)-Leu-OBu^{t}
(11) Z-Ile-Ser(Tos-Leu-OBu^{t}
(14) Z-Ile-Ser-Leu-OMe
(15) Nps-Ile-Ser-Leu-OMe
(18a) Z-Ala-Ile-Ser-Leu-Obu^{t} Ph$_3$C-Ala-OMe (S)
(18b) Z-D-Ala-Ile-Ser-Leu-OBu^{t} Ph$_3$C-Ala-OMe (S)
(23) Z-D-Ala-Ile-Ser(Dpp)-Leu Ph$_3$C-Ala-OMe (S)
(25) Z-D-Ala-Ile-Ser-Leu-OMe Ph$_3$C-Ala-OMe (S)

Compound [a]	Acylating component (mmol)	Amino-component (mmol)	Yield [b] (%)	M.p./°C	[α]D (°)	T/°C	c (solvent)	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(3)	Nps-Ser [c] (18.5)	Leu-OBu^{t} (16.7)	63	106—109 [d]	−88.6	20	1.4 (MeOH)	(7), 0.6	53.4	6.9	9.7	$C_{19}H_{29}N_3O_6S$	53.4	6.8	9.8
(7)	Z-Ile [e] (7.5)	Ser-Leu-OBu^{t} [f] (4.9)	90	126—128 [g]	−14.5	22	2 (EtOAc)		62.15	8.6	8.0	$C_{27}H_{43}N_3O_7$	62.2	8.3	8.1
(8)	Z-Ile (1.4)	Ala(3Cl)-Leu-OBu^{t} [h] (0.9)	72	125—128 [d]				(1), 0.6	59.9	7.85	7.5	$C_{27}H_{42}ClN_3O_6$	60.0	7.8	7.8
(11)	Z-Ile (7.5)	Cmpd. (5) [f] (5)	83	105—109 [i]	−10.7	22	2 (EtOAc)	(6), 0.8; (7), 0.7 (1), 0.5	59.9	7.3	6.3	$C_{34}H_{49}N_3O_9S$	60.4	7.3	6.2
(14)	Z-Ile (25)	Ser-Leu-OMe [j] (20.1)	71	177—179 [k]	−12.5	30	1 (DMF)		59.7	7.6	8.7	$C_{24}H_{37}N_3O_7$	60.1	7.8	8.8
(15)	Nps-Ile [l] (9)	Cmpd. (6) (9)	80	146—148 [d]	−33	30	1 (MeOH)	(6), 0.5	53.8 †	7.1 (S, 6.3)	11.25	$C_{22}H_{34}N_4O_7S$	53.0	6.9 (S, 6.4)	11.2
(18a) [m]			70	92—100 [d]	+10.4	25	3 (MeOH)	(1), 0.4; (9), 0.95	64.9 ‡	7.4 (S, 3.5)	7.3	$C_{53}H_{69}N_5O_{10}S$	65.75	7.2 (S, 3.3)	7.2
(18b) [n]			85	81—91 [o]	+19.9	30	3 (MeOH)	(6), 0.9; (7), 0.3 (1), 0.4	65.9	7.35 (S, 3.3)	7.1	$C_{53}H_{69}N_5O_{10}S$	65.75	7.2	7.2
(23)	Cmpd. (2b) [p] (2.25)	Cmpd. (13) (2.3)	68	97—107 [q]	+32.1	30	1 (DMF)	(6), 0.45; (10), 0.8	63.6	6.2 (P, 2.7; S, 2.9)	6.0	$C_{61}H_{70}N_5O_{13}PS$	64.0	6.2 (P, 2.7; S, 2.8)	6.1
(25)	Cmpd. (2b) [r] (0.55)	Cmpd. (16) (0.65)	65	167—169 [d]	+26.2	30	1 (MeOH)	(7), 0.3	64.6	6.9 (S, 3.6)	7.5	$C_{50}H_{63}N_5O_{10}S$	64.8	6.9 (S, 3.5)	7.6

* Abbreviations follow those given by the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature reprinted in 'Amino-acids, Peptides, and Proteins, vol. 4,' ed. G. T. Young, Specialist Periodical Reports, The Chemical Society, London, 1972, p. 441; also ⌐S¬ = lanthionine; Dha = dehydroalanine; —OPac = phenacyl Ala Ala ester; Dpp = diphenylphosphoryl; Ms = methanesulphonyl; DCC = dicyclohexylcarbodi-imide; DCHA = dicyclohexylamine; DMF = dimethylformamide; THF = tetrahydrofuran; all amino-acids are of the L-configuration except for the D-hemilanthionine which is indicated. † The higher carbon value can be attributed to some minor contamination from retained solvent (light petroleum). ‡ In our experience, lower carbon values are not unusual for compounds bearing a triphenylmethyl group.

[a] General procedures are described in the Experimental section. All the compounds are new and have been synthesized to be used as intermediates in the synthesis of the cyclopeptides (A) and (B). Some of them have been proved especially useful as shown in Schemes 1 and 3. [b] Yields are for product with the stated constants and analysis. [c] Liberated from its dicyclohexylammonium salt [11b] and coupled with the amino-component by the DCC method in THF (75 ml). [d] From EtOAc–light petroleum. [e] Coupled with the amino-component by the mixed anhydride method. [f] The ester free base was liberated from its hydrochloride {prepared by hydrogenolysis of Z-Ser-Leu-OBu^{t} [12] and recrystallized from isopropyl alcohol: m.p. 180—182 °C, $[\alpha]_D^{20}$ −31° (*c* 2, MeOH), R_F (8) 0.8} by addition of Et_3N (0.68 ml) in THF (15 ml). [g] From MeOH–water (1 : 1). [h] Z-Ala(3Cl)-Leu-OBu^{t} was prepared by coupling Z-Ala(3Cl), DCHA [13] (1 g, 2.3 mmol), and HCl, Leu-OBu^{t} [14] (0.5 g, 2.3 mmol) in $CHCl_3$ (10 ml) with DCC (0.5 g) at 0 °C; recrystallized from acetone–water, yield 82%, m.p. 77—80 °C, R_F (1) 0.65. The protected dipeptide was hydrogenolysed to give the hydrochloride [recrystallized from EtOH–Et_2O, m.p. 150 °C, R_F (1) 0.5, from which the ester free base was liberated *f*. [i] From $CHCl_3$–light petroleum. [j] Liberated (see under *f*) from the peptide hydrochloride (6). [k] From MeOH. [l] Coupling of Nps-Ile, DCHA [11a] with the peptide hydrochloride (6) in $CHCl_3$ (40 ml) was performed by DCC (2.1 g, 10 mmol) for 30 min at 0 °C and then for 5 h at room temperature. The crude product was extracted from the precipitate with acetone, the solution evaporated, and the residue dissolved in chloroform and purified on a silica gel (42 g) column eluted with EtOAc. [m] See Experimental section. [n] Prepared from compound (2b) analogously to compound (18a). [o] From Et_2O–light petroleum. [p] Coupling proceeded overnight at room temperature with the amino-component liberated from its hydrochloride in DMF (5 ml) by addition of *N*-methylmorpholine (0.28 ml, 2.35 mol). Water was added, the mixture extracted with EtOAc and washed with water, 10% citric acid, and water. The crude product was precipitated with ether–light petroleum. [q] From dichloromethane–ether–light petroleum. [r] Coupling *etc.* was performed as given under *n*. After evaporation of the solvent the mixture was extracted with EtOAc, washed with water, 10% citric acid, 2N-ammonium hydroxide, and water (to pH 7), and dried.

petroleum precipitated out the *product*. Yield and physical data are given in Table 2.

N$^{\alpha}$-*Benzyloxycarbonyl*-D-*hemilanthionyl*-L-*isoleucyl-dehydroalanyl*-L-*leucine* L-*hemilanthionine* α'-*Methyl Ester Hydrochloride* (29).—*cf.* Table 2.

N$^{\alpha}$-*Benzyloxycarbonyl*-D-*hemilanthionyl*-L-*isoleucyl-dehydroalanyl*-L-*leucyl*-L-*hemilanthionine* α'-*Methyl Ester* (B). —To a solution in dimethylformamide (40 ml) of compound (29) (558 mg, 0.81 mmol) cooled to −10° were added successively (*a*) triethylamine (1.14 ml; 10% in dimethylformamide), (*b*) 1-hydroxy-benzotriazole [120 mg; in dimethylformamide (2 ml)], and (*c*) dicyclohexylcarbodiimide (2 ml; 8.5% in dimethylformamide). The mixture was set aside for 1 h at −10 °C and then at 4 °C. During the next 4 d four portions (each 2 ml) of solution (*c*) were added, and after three more days the mixture was evaporated. The residue was triturated with tetrahydrofuran (40 ml), some dicyclohexylurea was filtered off, and to the filtrate acetic acid (1 ml, 10% in water) was added and the mixture left overnight at 4 °C. Urea was again pre-

TABLE 2

Partially protected peptides *

(5) Ser(Tos)-Leu-OBu^{t}
(6) Ser-Leu-OMe
(12) Ile-Ser-Leu-OBu^{t}
(13) Ile-Ser-(Dpp)Leu
(16) Ile-Ser-Leu-OMe
(17) Z-Ala-OPac—S—Ala-OMe
(19) Z-Ala-Ile-Ser-Leu-OBu^{t}—S—Ala-OMe
(20) Z-Ala-Ile-Ser-Leu—S—Ala-OMe
(22) Z-D-Ala-Ile-Ser(Dpp)-Leu-OBu^{t}—S—Ala-OMe
(24) Z-D-Ala-Ile-Ser(Dpp)-Leu—S—Ala-OMe
(27) Z-D-Ala-Ile-Dha-Leu-OMe—S—Ph_3C-Ala-OMe
(28) Z-D-Ala-Ile-Dha-Leu—S—Ph_3C-Ala-OMe
(29) Z-D-Ala-Ile-Dha-Leu—S—Ala-OMe
(30) Ala-Ile-Ser-Leu-Ala-OMe

Compound [a]	Starting material	Yield [b] %	M.p./°C	[α]D (°)	T/°C	c (solvent)	R_F (t.l.c.)	Found (%) C	H	N	Cl	Formula	Required (%) C	H	N	Cl
(5) [c]	(4) [d] (in (EtOAc)	93	126—128 [e]	−15	30	1 (MeOH)	(1), 0.3	51.45	7.2	6.0		$C_{20}H_{33}ClN_2O_6S$	51.7	7.12	6.0	
(6) [c]	Z-Ser-Leu-OMe [f,g]	84	122—125 [h]	−11.5	30	1 (MeOH)	(6), 0.15; (10), 0.6; (8), 0.65	44.8	7.75	10.6	13.7	$C_{10}H_{21}ClN_2O_4$	44.7	7.9	10.4	13.2
(12) [c]	(7) [g]	85	184—186 [e]	−26.9	23	2 (MeOH)	(8), 0.7	53.5	9.1	9.7	8.5	$C_{19}H_{38}ClN_3O_5$	53.8	9.0	9.9	8.4
(13) [i]	(9) [j]	97	187—189	+13.6	30	1 (DMF)	(7), 0.0; (8), 0.7	50.4	6.1 (Br, 12.5;	6.6 P, 4.5		$C_{27}H_{39}BrN_3O_6P$	50.3	6.1 (Br, 12.4,	6.5 P 4.8)	
(16) [c]	(15) [d] (in MeOH)	93	190—198 [h]	−18.9	30	1 (MeOH)	(6), 0.15; (10), 0.45; (8), 0.6; (3), 0.55	50.2	8.5	10.9	9.3	$C_{16}H_{32}ClN_3O_5$	50.3	8.45	11.0	9.3
(17) [c]	Z-Ala-OPac [k] —S— Ph_3C-Ala-OMe	57	96—99 [l]	−19.7	18	2 (MeOH)	(1), 0.1	54.0	5.7 (S, 6.11)	5.15	6.4	$C_{35}H_{37}ClN_2O_7S$	54.1	5.3 (S, 6.3)	5.5	6.9
(19) [c,m]	(18a)	96	158—160	−43.0	25	3 (MeOH)	(9), 0.85	52.1	7.35 (S, 4.2;	8.7 H_2O, 2.4)	4.6	$C_{34}H_{56}ClN_5O_{10}S$, H_2O	52.3	7.5 (S, 4.1;	9.0 H_2O, 2.3)	4.5
(20) [m]	(19)	70	194—199				(9), 0.6	53.5	7.2	10.3		$C_{30}H_{47}N_5O_{10}S$	53.8	7.1	10.5	
(22) [c]	(21) [n]	91	146—149 [o]	−13.4	30	2 (MeOH)	(6), 0.6; (8), 0.85; (3), 0.75	55.7	6.75	7.1		$C_{46}H_{65}ClN_5O_{12}PS$	55.55	6.6	7.0	
(24) [c]	(23) [p]	56	119—122	+3.8	30	1 (DMF)	(8), 0.65; (3), 0.6	54.1	6.3 (P, 3.4)	7.6		$C_{42}H_{57}ClN_5O_{13}PS$	53.8	6.1 (P, 3.3)	7.5	
(27) [m]	(26)	84	100—115 (decomp.)	+30.6	30	1.2 (MeOH)	(7), 0.65	65.85	6.9 (S. 3,4)	7.6		$C_{50}H_{61}N_5O_9S$	66.1	6.8 (S, 3.5)	7.7	
(28) [m]	(27)	90	94—104	+41.5	30	1 (DMF)	(7), 0.4; (8), 0.75; (11), 0.8	65.4	6.55 (S, 3.5)	7.7		$C_{49}H_{59}N_5O_9S$	65.8	6.65 (S, 3.6)	7.8	
(29) [c]	(28) [n]	95	108—118 [h]	+1.1	30	2 (DMF)	(6), 0.2; (5), 0.65; (11), 0.55; (3), 0.75	51.8	6.8 (S, 4.8)	9.9	5.3	$C_{30}H_{46}ClN_5O_9S$	52.4	6.7 (S, 4.7)	10.2	5.15
(30) [q,m]	(A)	37	250—255				(9), 0.8	44.8	5.7	14.8		$C_{38}H_{42}N_8O_{14}S$	45.0	5.7	15.0	

* Abbreviations as for Table 1.

[a,b] As Table 1. [c] As hydrochloride. [d] For the removal of the *N*-Nps group see ' General Procedures.' [e] From EtOH–Et_2O. [f] Prepared as an oil by coupling Z-Ser-N_3 with Leu-OMe. [g] Removal of the *N*-Z group by hydrogenolysis (see ' General Procedures '). [h] From MeOH–Et_2O. [i] As hydrobromide. [j] Removal of *N*-Z and the OBu groups with HBr–AcOH (see ' General Procedures '). [k] *N*$^{\alpha'}$-Benzyloxycarbonyl-*N*$^{\alpha}$-trityl-L-lanthionine α'-methy ester [1] (3 g, 5 mmol) was treated with Et_3N (0.7 ml) and phenacyl bromide [15] (1 g) in EtOAc (12 ml) at 0 °C. Next day, the triethylamine salt was removed, the filtrate washed repeatedly with water, evaporated, and the oil residue dissolved in MeOH–EtOAc (10 : 1; 55 ml), treated with HCl–MeOH (4.5N; 1.25 ml) overnight, and evaporated. The oily hydrochloride was solidified on trituration with light petroleum and then with EtOAc. [l] From isopropyl alcohol. [m] See Experimental section. [n] Detritylation and isolation of the hydrochloride as described for compound (19). [o] From $CHCl_3$–Et_2O and $CHCl_3$–light petroleum. [p] Detritylation of compound (23) (1.12 mmol) was performed in acetic acid (5 ml) with a solution of concentrated hydrochloric acid in acetic acid (0.48N; 4.3 mmol). After 30 min the hydrochloride (24) was precipitated with ether and recrystallized twice from DMF–H_2O. [q] As picrate.

TABLE 3

O-Substituted serine peptides *

(4) Nps-Ser(Tos)-Leu-OBu^t
(9) Z-Ile-Ser(Dpp)-Leu-OBu^t
(10) Z-Ile-Ser(Ms)-Leu-OBu^t
(21) Z-D-Ala-Ile-Ser(Dpp)-Leu-OBu^t (S) Ph$_3$C-Ala-OMe
(26) Z-D-Ala-Ile-Ser(Dpp)-Leu-OMe (S) Ph$_3$C-Ala-OMe

Compound [a]	Starting material	Yield [b] (%)	M.p. (°C)	[α]D (°)	[α]D T/°C	[α]D c (solvent)	R_F (t.l.c.)	Found (%) C	Found (%) H	Found (%) N	Formula	Required (%) C	Required (%) H	Required (%) N
(4)	(3) [c]	92	106—110 [d]	−11.4	30	1 (MeOH)	(7), 0.9	53.6	6.1	7.2	$C_{28}H_{35}N_3O_8S_2$	53.7	6.1	7.2
(9)	(7) [e]	76	143—144 [f]	−18.3	20	1 (DMF)	(1), 0.55	61.8	7.0 (P, 4.0)	5.45	$C_{39}H_{53}N_3O_{10}P$	62.1	6.95 (P, 4.1)	5.6
(10)	(7) [g]	54	127—128 [h]				(7), 0.45	56.7	7.6 (S, 5.1)	6.9	$C_{28}H_{45}N_3O_9S$	56.1	7.6 (S, 5.35)	7.0
(21)	(18b) [e]	76	98—102 [i]	+18.3	30	3 (MeOH)	(7), 0.75; (1), 0.5	65.1	6.6 (S, 2.8)	5.8	$C_{65}H_{76}N_5O_{13}PS$	65.0	6.55 (S, 2.7)	5.8
(26)	(25) [j]	83	105—115 (decomp.)	+29.4	30	1 (DMF)	(7), 0.6	64.1	6.5 (P, 2.6; S, 2.7)	6.1	$C_{69}H_{72}N_5O_{13}PS$	64.3	6.3 (P, 2.7; S, 2.8)	6.05

* Abbreviations as for Table 1.

[a, b] As Table 1. [c] Dissolved (15 mmol) in dry pyridine (30 ml) at −10 °C and treated with TosCl (30 mmol) for 1 h at 0 °C and for 3 h at room temperature. The mixture poured onto ice, extracted in EtOAc–Et_2O, washed with water, 10% citric acid, and water, dried, and evaporated. [d] From CCl_4. [e] Added (5 mmol) to Dpp-Cl (10 mmol) dissolved in dry pyridine (10 mmol) at −15 °C. After 4 h at 0 °C the mixture poured onto ice and the solid product filtered off. [f] From $CHCl_3$–light petroleum and then from isopropyl alcohol. [g] As under [e] except for: CH_3SO_2Cl, 1 h at 0 °C and 3 h at room temperature. [h] From EtOAc–Et_2O. [i] From DMF–H_2O and then from EtOAc–light petroleum. [j] As described in the Experimental section.

cipitated, removed by filtration, the filtrate evaporated, and the residue treated with methanol. The product isolated by filtration was treated with hot tetrahydrofuran and left overnight at 4 °C. The crude product (82 mg) was dissolved in dimethylformamide (2 ml) and purified on a Sephadex LH-20 column (100 g) eluting with the same solvent (35.5 ml h^{-1}). Fractions (from 216—239 ml) containing pure product were combined and evaporated to *ca.* 3 ml. Addition of ether gave compound (B) (57 mg), m.p. 252—253 °C, $[\alpha]_D^{30}$ −63.4 (*c* 1 in dimethylformamide), R_F (6) 0.7, R_F (10) 0.8 (Found: C, 56.7; H, 7.0; N, 10.9; S, 5.0. $C_{30}H_{43}N_5O_8S$ requires C, 56.7; H, 6.8; N, 11.05; S, 5.1); amino-acid analysis: Ile, 1.0; Leu 1.02; *meso*-lanthionine + lanthionine 1.03; NH_3, 0.85 (corrected); $\nu_{max.}$ (KBr disc) at 3 100, 1 635, and 895 cm^{-1}. Molecular weight determination (osmometrically in dimethylformamide solution, in two concentrations): 648, 644 (calc. 633.8).

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