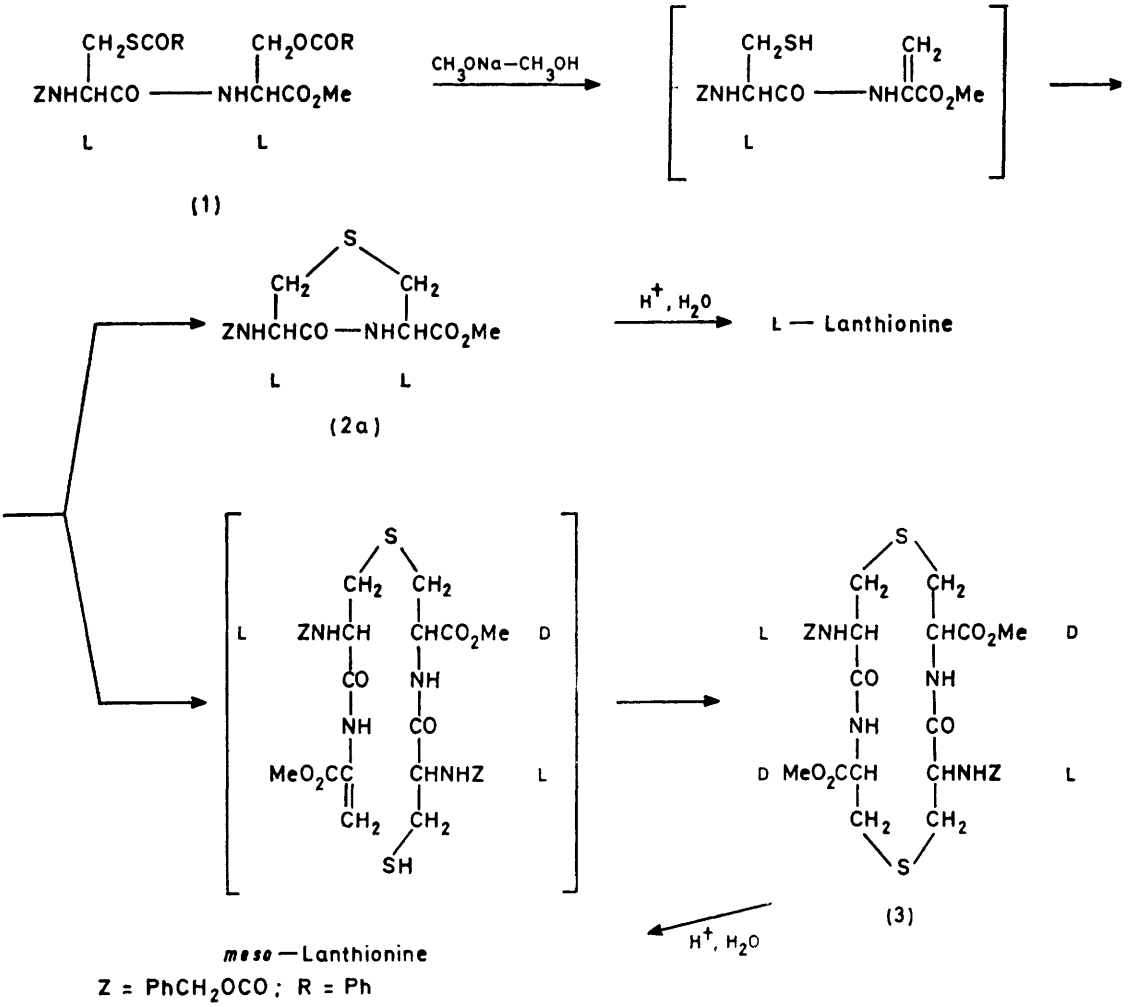
Lanthionine Chemistry. Part 4.[1] Synthesis of Diastereoisomeric Cyclolanthionyl Derivatives

By **Iphegenia Photaki,* Ioannis Samouilidis, Stephanes Caranikas,** and **Leonidas Zervas,*** Laboratory of Organic Chemistry, The University of Athens, Athens 144, Greece

The direct synthesis of monomeric *cyclo*-L-lanthionyl (2a) and *cyclo*-(D→L)-lanthionyl (2b) derivatives has been achieved using derivatives of L- or *meso*-lanthionine differentially protected at the amino and the carboxyl groups, *e.g.* N^{α}-benzyloxycarbonyl-$N^{\alpha'}$-trityl-L- (or D→L)-lanthionine α'-methyl ester (6a, b). The specific rotation of L-lanthionine in acidic or alkaline solutions is markedly temperature-dependent; it increases in the former and decreases in the latter as the temperature increases. Previous investigations on the reversibility of the specific rotation change in acidic solutions have been confirmed and expanded. After heating acidic solutions of L-lanthionine above 96 °C, or at this temperature for >5 h, an irreversible change of the specific rotation was observed. Racemization calculated from the observed decrease of the specific rotation includes transformation to both DL- and *meso*-lanthionine. The *meso*-stereoisomer was determined by Moore and Stein amino-acid analysis. After acidic hydrolysis the *cyclo*-L-lanthionyl compound (2a) exhibited a similar temperature- and time-dependent formation of DL- and *meso*-lanthionine. A change of the configuration was also observed when either *meso*-lanthionine or *cyclo*-(D→L)-lanthionyl derivative (2b) were treated under acidic conditions.

In the course of earlier work on the temporary protection of SH and OH groups during peptide synthesis, attempts to remove the *S*- and *O*-benzoyl groups from the neighbouring cysteine and serine moieties of the dipeptide derivative (1) led to the isolation and the identification of the monomer *cyclo*-L-lanthionyl (2a) and the dimer *cyclo*-(L→D)-lanthionyl (3) derivatives (Scheme 1) [1b] out of a mixture of other cyclolanthionyl diastereoisomers (of various molecular sizes) probably formed.

In this paper we describe the direct synthesis of the



Scheme 1

diastereoisomeric *cyclo*-L-lanthionyl (2a) and *cyclo*-(D→L)-lanthionyl (2b) derivatives (Scheme 2) by methods which we also require for the synthesis of lanthionine-containing *cyclo*-peptides resembling those which occur naturally.[2]

RESULTS AND DISCUSSION

The condensation of *N*-benzyloxycarbonyl-L-cysteine with β-chloro-L- or -D-alanine (Scheme 2) in aqueous alkaline solution under nitrogen, led to the isolation of monobenzyloxycarbonyl lanthionines (4a or c) in moderate yields. Much better yields of compound (4a) or its diastereoisomer (4b) have been obtained by condensation of *N*-benzyloxycarbonyl-β-L- or -D-chloroalanine with L-cysteine in a two-phase system of tetrahydrofuran and aqueous alkaline solution using benzyltrimethylammonium hydroxide (Triton B) as a phase transfer catalyst.[3] It should be noted that owing to the differential protection of α-amino-groups, the two asymmetric α-carbon atoms of *meso*-lanthionine are unsymmetrically substituted. Therefore derivatives of *meso*-lanthionine such as *e.g.* (4b) and (4c) are enantiomers.*

Monobenzyloxycarbonyl-L- and -(D→L)-lanthionine (4a, b) were transformed by known methods of esterification,[4] tritylation,[5] and partial saponification to N^{α}-benzyloxycarbonyl-$N^{\alpha'}$-trityl-L- and -(D→L)-lanthionine α′-methyl esters (6a or b). Because of the steric hindrance exerted by the α-*N*-trityl group, the neighbouring α-carboxyl ester group is resistant to saponification [5] and has been preserved in compounds (6a, b). Transformation to the *p*-nitrophenyl esters [6] (7a, b), followed by detritylation [5] and cyclization [7] of these active esters in the presence of hydroxybenzotriazole [8] led to the cyclolanthionyl derivatives (2a) and (2b). Using an alternative method the cyclic compound (2a) was obtained by detritylation of compound (6a) and cyclization using dicyclohexylcarbodi-imide [9] in the presence of *N*-hydroxysuccinimide.[10]

The *cyclo*-L-lanthionyl derivative (2a) prepared as above proved to be identical with that prepared indirectly [1a,b] as shown in Scheme 1. On the other hand the monomeric structure of the *cyclo*-(D→L)-lanthionyl derivative (2b) was demonstrated by molecular-weight determination. In the case of compound (2a) the free amino-acid *cyclo*-L-lanthionyl (10) could also be obtained, in very good yield, by saponification and debenzyloxycarbonylation.

Amino-acid analysis of the *cyclo*-L-lanthionyl derivative (2a) [1b] after acid hydrolysis showed the presence of variable amounts of *meso*-lanthionine. This is in agreement with the observation of Gross and Morell [2a] that 'L-lanthionine when exposed to the conditions of standard acid hydrolysis is transformed to *meso*-lanthionine and DL-lanthionine'. The above findings, prompted us to study and to prove the temperature-dependence of the specific optical rotation of L-lanthionine in acidic solutions.[1b] Table 1 contains new and more accurate data obtained in both acidic and alkaline conditions.†

TABLE 1

Temperature-dependence of the specific optical rotation of L-lanthionine at 589 nm

(*a*) In 6N HCl (*c* 5)

θ_c/°C	7	20	25	30	40	50
$[\alpha]_D$	−0.7	+1.4	+2.9	+4.2	+6.5	+8.8
θ_c/°C	60	70				
$[\alpha]_D$	+10.7	+12.1				

(*b*) In 2.4 N NaOH (*c* 5)

θ_c/°C	7	15	23	35	40	45
$[\alpha]_D$	+13.7	+9.7	+7.7	+3.2	+1.8	+0.24
θ_c/°C	50	55	60	70		
$[\alpha]_D$	−1.1	−2.3	−3.5	−5.7		

Since L-lanthionine can be considered as an *S*-substituted L-cysteine derivative, the great temperature dependence of its optical rotation in acidic solution can be compared with the similar behaviour of some other thioether derivatives of L-cysteine reported in the literature.[12]

The change of the molecular optical rotation of L-lanthionine in 6N hydrochloric acid is approximately linear over the temperature range studied and the calculated temperature coefficient $\Delta[M]_D/\Delta T$ is positive (0.4—0.5) and comparable with those of the thioethers mentioned above.[12a]

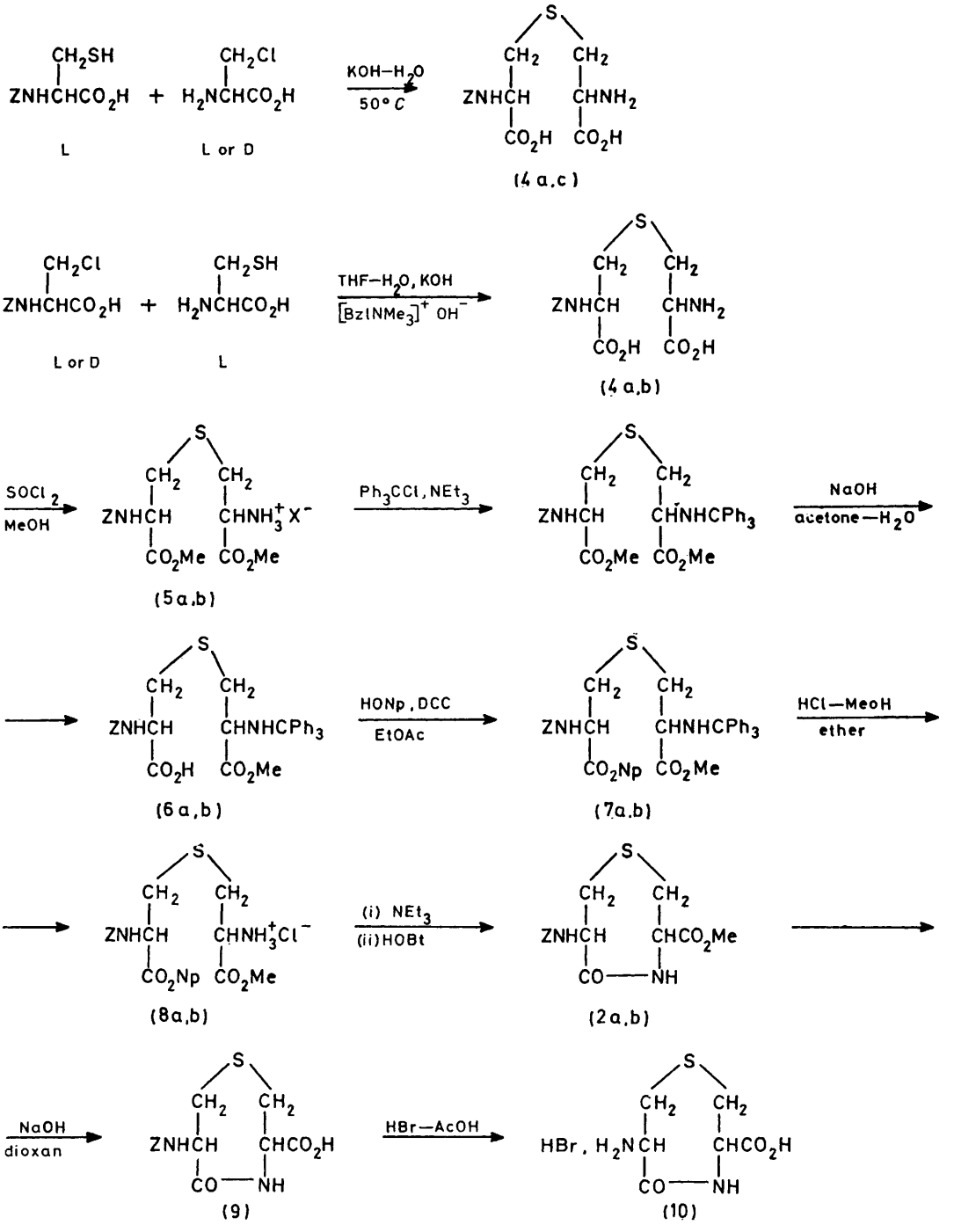
What is more important is our observation [1b] that changes in the optical rotation in acidic solutions heated to *ca.* 96 °C for 5 h, are reversible, whereas heating either above 96 °C, or at this temperature but for a longer period of time, results in optical inactivation, *i.e.* racemization of L-lanthionine [in this case 'racemization' includes the transformation of L-lanthionine to either of the diastereoisomeric forms racemic (DL) or *meso*-lanthionine]. We have now examined the percentage of *meso*-lanthionine ‡ produced after heating L-lanthionine under acidic conditions at different temperatures for varying periods of time. These results are shown in Table 2. As with the optical inactivation, the percentage of *meso*-lanthionine increases with the temperature and/or the duration of heating. A comparison of the optical rotation data [1b] with the data of Table 2 shows that above the critical temperature of 96 °C, *i.e.* at 110 or 120 °C, the racemization (calculated from the observed optical inactivation) was larger than the percentage of *meso*-lanthionine produced, indicating the parallel formation of some D-lanthionine.

Table 3 contains the results of the amino-acid analysis after acidic hydrolysis of *cyclo*-L-lanthionyl (10) and its

* Compounds (4b) and (4c) can be named *N*-benzyloxycarbonyl-*S*-(L-β-amino-β-carboxyethyl)-D-cysteine and *N*-benzyloxycarbonyl-*S*-(D-β-amino-β-carboxyethyl)-L-cysteine respectively. However we find it easier to designate them as N^{α}-benzyloxycarbonyl-(D→L)-lanthionine and N^{α}-benzyloxycarbonyl-(L→D)-lanthionine respectively [*cf.* Scheme 2, (4b) and (4c)]. The arrow gives the direction of the peptide bond (which will be formed by the cyclisation) in the usually accepted sense.

† The temperature dependence of the specific rotation in alkaline solutions was known [11] for two temperatures (+4 and +22 °C).

‡ In the Moore and Stein analysis, *meso*-lanthionine is separated from D- and L-lanthionine which, being enantiomeric, elute together.



SCHEME 2 a = L, b = (D→L) c = (L→D), X^- = a, chloride, b, oxalate monoanions, HOB = *N*-hydroxybenzotriazole, Bzl = $PhCH_2$, DCC = dicyclohexyldi-imide, HONp = *p*-nitrophenol

TABLE 2

Moore and Stein amino-acid analyses after heating acidic solutions of L-lanthionine

Heating conditions			Lanthionine	*meso*-Lanthionine
θ_c/°C	*t*/h	Acid	(%)	(%)
20			100	0
96	5	*a*	97.8	2.2
96	15	*a*	95	5
110	6	*a*	92.1	7.9
110	24	*a*	75.8	24.2
110	48	*a*	63.2	36.8
110	72	*a*	56.7	43.3
110	168	*a*	50.4	49.6
20		*b*	100	0
60	5	*b*	100	0
20		*b*	100	0
96	5	*b*	98	2
96	15	*b*	93.3	6.7
96	25	*b*	90.4	9.6
96	45	*b*	85.3	14.7

[a] 6N HCl. [b] Acetic acid–concentrated HCl (1 : 1).

two derivatives (2a) and (9). It can be seen that the formation of *meso*-lanthionine from (2a) shows a similar temperature- and time-dependence as from L-lanthionine.

As expected, pure *meso*-lanthionine and the *cyclo*-(D→L)-lanthionyl derivative (2b) showed racemization of the optical centres as revealed by amino-acid analysis after treatment with hot, strong acid (results not detailed here).

α-proton sufficiently acidic to allow acid enol formation, resulting in racemization. In our opinion, a more detailed study of the kinetics of racemization at different temperatures could give an answer to the problem.

In conclusion, we note that during synthetic work using open or cyclic L-lanthionine compounds, attention must be given to the applied temperature conditions in order to avoid changes in the configuration of the products. Moreover, the results on the content of lanthionine diastereoisomers after acidic hydrolysis of lanthionine-containing peptides should also be interpreted in the light of the above results.

EXPERIMENTAL

M.p.s were taken for samples in capillary tubes. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter (1-dm cell). Anhydrous solvents were used for the coupling reactions. Evaporations were carried out under reduced pressure at 35—40 °C. When necessary, solutions in organic solvents were dried over sodium sulphate. Before analysis, compounds were dried over P_2O_5 at room temperature under high vacuum; elemental analysis and the water content determination by the Karl Fischer method were performed at the Analytical Laboratory of F. Hoffmann–La Roche and Co. Basle, Switzerland, under the direction of Dr. A. Dirscherl. The molecular-weight determination was performed at the Analytical Laboratory

TABLE 3

Moore and Stein amino-acid analyses after acidic hydrolysis of *cyclo*-L-lanthionyl derivatives

Hydrolysed compound	Acid	Hydrolysis conditions θ_c/°C	*t*/h	Lanthionine (%)	*meso*-Lanthionine (%)
(2a) [a]	*b*	100	5	90.2	9.8
(2a) [a]	*b*	110	24	73.0	27.0
(2a) [a]	*b*	110	36	65.3	34.7
(2a) [a]	*b*	110	72	52.7	47.3
(2a) [a]	*b*	110	144	51.4	48.6
(2a) [c]	*d*	100	5	96.3	3.7
(2a) [a,c]	*d*	96	5	97.3	2.7 [e]
(2a) [a]	*d*	96	15	93.2	6.8
(2a) [c]	*d*	96	15	93.0	7.0
(2a) [c]	*d*	60	5	99.5	0.5
(2a) [c]	*d*	60	15	99.1	0.9
(2a) [a]	*d*	60	5	100.0	0.0
(2a) [a]	*d*	60	15	100.0	0.0
(9) [c]	*d*	60	5	100.0	Trace
(9) [c]	*d*	60	15	98.9	1.1
(10) [c]	*d*	60	5	100.0	0.0
(10) [c]	*d*	60	15	100.0	Trace

[a] Compound (2a) prepared indirectly.[1b] [b] 6N HCl. [c] Compound (2a) prepared by direct synthesis (this paper). [d] Acetic acid–concentrated HCl (1 : 1). [e] Lanthionine isolated from a similar experiment was shown to contain about 7% of optically inactive lanthionine.[1b]

Bláha *et al.*[12a] have interpreted the reversible high temperature dependence † of the specific rotation of some *S*-substituted L-cysteine derivatives on the assumption that the sulphur substituent in the β-position acts as an additional optically active chromophore whose contribution is strongly dependent on temperature. Jacobson *et al.*[12b] give an explanation for the racemization of *e.g.* *S*-(2,4-dinitrophenyl)-L-cysteine in refluxing 6N HCl, assuming that the inductive effect of the positively charged sulphur atom at the β-position renders the

† The highest temperature applied by these authors, as shown from the given results, was *ca.* 90 °C.

of A. Bernhardt, Elbach, Germany. Amino-acid analyses were performed on samples of lanthionine or its derivatives after heating or hydrolysis in evacuated tubes. The analyses were carried out at the Biochemistry Department, Mount Sinai School of Medicine, New York, using a Beckman–Spinco amino-acid analyser (model 120C) according to the method of Spackman *et al.*[13] For paper electrophoresis, an LKB-3276 apparatus was used, with Schleicher and Schüll 2043-B paper. R_F Values refer to t.l.c. on silica gel G (Fluka) containing 13% calcium sulphate in the following solvent systems (proportions by volume): (1) toluene–pyridine–acetic acid (80 : 10 : 1); (2) methanol–chloroform–acetic acid–pyridine (80 : 10 : 1 : 5); (3) butan-

1-ol–acetic acid–water (4 : 1 : 1); (4) butan-1-ol–acetic acid–water–pyridine (30 : 6 : 24 : 20); (5) methanol–pyridine (9 : 1); (6) chloroform–methanol (9 : 1); and (7) carbon tetrachloride–ethyl acetate (1 : 1). Plates were developed with ninhydrin solution [0.5% in acetone–acetic acid–water (90 : 5 : 5)], with iodine or, in the case of the cyclo-compounds, with rhodamine B solution (1% in ethanol) under a u.v. lamp.

Our preparations of lanthionine or its *N*-benzyloxycarbonyl derivatives showed a negative cysteine (cystine) nitroprusside test after sodium cyanide treatment of the compounds dissolved in ammonium hydroxide.

L-Lanthionine (yield 75%) was prepared according to the literature [11] but starting from L-cysteine, instead of L-cystine, and β-chloro-L-alanine. By the same method and using β-chloro-D-alanine, *meso*-lanthionine (yield 55%) was also prepared.

N$^{\alpha}$-*Benzyloxycarbonyl*-(L→D)-*lanthionine* (4c).—*N*-Benzyloxycarbonyl-L-cysteine [14] (2.35 g, 9.2 mmol) was dissolved under nitrogen in water (5 ml) containing potassium hydroxide (1.4 g, 25 mmol). To the solution, heated at 50 °C, β-chloro-D-alanine hydrochloride [15] (1 g, 6.25 mmol) and potassium hydroxide (1.15 g, 20 mmol) in water (20 ml) were simultaneously added during 1 h. Stirring under nitrogen was continued for 3 h at room temperature. The mixture was diluted with water (5 ml) and acidified with 5N hydrochloric acid to pH 1 at 25 °C. Unreacted *N*-benzyloxycarbonyl-L-cysteine was extracted twice into ether. After removal of the organic solvent under reduced pressure 4N potassium hydroxide was added to the aqueous layer, to pH 3.1. After 2 d at 4 °C, the precipitated crystalline product was collected by filtration, washed with water, and recrystallized from water–methanol (3 : 7, v/v) to give the *product* (0.8 g, 37%), m.p. 187—188 °C (decomp.); $[\alpha]_D^{19}$ −23.3° (*c* 3 in 1N-HCl); R_F(3) 0.5; R_F(4) 0.5; amino-acid analysis after hydrolysis with concentrated hydrochloric acid–acetic acid (1 : 1) for 5 h at 60 °C: *meso*-lanthionine 100%; lanthionine, trace; at the same conditions for 15 h: *meso*-lanthionine 97.9%; lanthionine 2.1% (Found: C, 48.9; H, 5.3; N, 8.1. $C_{14}H_{18}N_2O_6S$ requires C, 49.1; H, 5.3; N, 8.2%).

N$^{\alpha}$-*Benzyloxycarbonyl*-(D→L)-*lanthionine* (4b).—(*a*) To a solution of potassium hydroxide (1 g) in water (3 ml) through which nitrogen had been bubbled for 10 min, L-cysteine hydrochloride (0.95 g, 6 mmol) was added. (*b*) At the same time a suspension of *N*-benzyloxycarbonyl-β-chloro-D-alanine dicyclohexylammonium salt (1.33 g, 3 mmol) [16] in ether was shaken in a separatory funnel with 0.2N sulphuric acid until it dissolved. The organic layer was washed with water (3 ×), dried, and evaporated. The oily residue was dissolved in tetrahydrofuran (10 ml) and added under nitrogen and with vigorous stirring to the solution from (*a*). Triton B [3a] (0.13 ml) was added and the mixture was heated at 70 °C under reflux for 5 h. Treatment of the mixture as under (4c) gave (at pH 2.7) a *solid* which was filtered off and washed with water, ethanol, and ether, yield 0.68 g (68%), m.p. 200—201 °C (decomp.) after recrystallization from water–methanol (1 : 4); $[\alpha]_D^{19}$ +23.1° (*c* 3 in 1N-HCl); R_F the same as for compound (4c) [Found: C, 49.5; H, 5.4; N, 8.3; S, 9.6. Required C, H, N as for (4c); S, 9.4%].

N$^{\alpha}$-*Benzoyloxycarbonyl*-L-*lanthionine* (4a).—(*a*) This was prepared by the method described for compound (4c) except that precipitation occurred at pH 2.8 and the mixture used for recrystallization was methanol–water (3 : 2), yield 1 g (46%), m.p. 192—193 °C; $[\alpha]_D^{25}$ −22.7°; $[\alpha]_D^{20}$ −24.3°; $[\alpha]_D^{17}$ −25.1°; $[\alpha]_D^{7}$ −27.8° (*c* 2 in 1N-HCl); R_F (2) 0.6; R_F (3) 0.6; R_F (5) 0.7; amino-acid analysis after hydrolysis with concentrated hydrochloric acid–acetic acid (1 : 1) for 5 h at 60 °C: lanthionine 99.3%; *meso*-lanthionine 0.7%; at the same conditions for 15 h: lanthionine 98.4%; *meso*-lanthionine 1.6% (Found: C, 48.8; H, 5.4; N, 8.0; S, 9.3%).

(*b*) The title compound was also prepared as described for (4b), yield 75%.

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-L-*lanthionine Bisdiethylammonium Salt*.—*N*-Benzyloxycarbonyl-L-lanthionine (4a) (1.7 g, 5 mmol) was dissolved in water (2 ml), diethylamine (2 ml), and isopropanol (4 ml), and trityl chloride (1.8 g) was added with vigorous shaking in 12 portions during 1 h.[5] After a further 10 min stirring water (15 ml) was added and the mixture extracted twice with chloroform. The combined organic layer * was washed with water, dried, and evaporated to dryness. To the residual oil dissolved in ether a few drops of diethylamine were added and the precipitated solid was filtered off, triturated with ether, and recrystallized from acetone to give the title compound (0.4 g, 11%), m.p. 150—154 °C. To remove some unreacted *N*-benzyloxycarbonyl-L-lanthionine the diethylammonium salt was shaken in a separatory funnel with ether and cold 0.1N-sulphuric acid. The organic layer was repeatedly washed with water and dried; diethylamine was added and the precipitated salt was crystallized from acetone–ether, m.p. 154—156 °C; $[\alpha]_D^{29}$ +8.9° (*c* 2 in methanol); R_F (1) 0.15 (Found: C, 67.2; H, 7.6; N, 7.5; $C_{41}H_{54}N_4O_6S$ requires C, 67.4; H, 7.45; N, 7.7%).

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*lanthionine Dimethyl Ester Hydrochloride* (5a).—A solution of compound (4a) (6.3 g, 18.4 mmol) in methanol (35 ml) containing thionyl chloride (3.45 ml) was stirred for 2 h at room temperature and then set aside overnight. The solvent was removed under reduced pressure, the residue was twice dissolved in methanol and evaporated to dryness, and finally it was dissolved in the necessary amount of hot methanol and the hydrochloride precipitated with an equal volume of ether. It was left at 4 °C, filtered off, washed with ether and recrystallized from methanol containing hydrogen chloride, and then ether, yield 6.9 g (92%), m.p. 183 °C (decomp.); $[\alpha]_D^{24}$ −26.8° (*c* 2.3 in methanol); R_F (1) 0.1; R_F (5) 0.8; amino-acid analysis after hydrolysis with concentrated hydrochloric acid–acetic acid (1 : 1) for 5 or 15 h at 60°: lanthionine 100%; *meso*-lanthionine 0% (Found: C, 47.1; H, 5.7; N, 6.8; Cl, 9.0; S, 8.0; OMe, 15.0. $C_{16}H_{23}ClN_2O_6S$ requires C, 47.2; H, 5.7; N, 6.9; Cl, 8.7; S, 7.9; OMe, 15.25%).

N$^{\alpha}$-*Benzyloxycarbonyl*-(D→L)-*lanthionine Dimethyl Ester Oxalate* (5b).—The hydrochloride was prepared as an oil by the method described for (5a). The ester salt was taken up in ether and treated with a saturated solution of potassium hydrogencarbonate. After shaking, the phases were separated and the ethereal layer was washed with water to neutral pH, dried, and evaporated. The residual oil appeared homogeneous by t.l.c., R_F (6) 0.7. Attempts to prepare a crystalline hydrochloride were unsuccessful. To a cooled ethereal solution of the ester free base, a solution of oxalic acid [1 equiv. calculated on the *N*-benzyloxycarbonyl-(D→L)-lanthionine used] in ether was

* From the acidified (to pH 3) aqueous layer crystalline starting material (4a) (0.5 g, 30%) was isolated, m.p. 188 °C, $[\alpha]_D^{20}$ −24° (*c* 2 in 1N-HCl), R_F (1) 0.0.

added and the crystalline *oxalate* separated out. It was set aside overnight at 4 °C, filtered off, and recrystallized from methanol–ether (yield 61%), m.p. 156—157 °C after recrystallization from isopropanol; $[\alpha]_D^{30}$ +30.8° (*c* 1 in methanol) (Found: C, 46.9; H, 5.0; N, 6.1; S, 7.0. $C_{18}H_{24}N_2O_{10}S$ requires C, 46.95; H, 5.25; N, 6.1; S, 7.0%).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-L-*lanthionine* α′-*Methyl Ester* (6a).—(a) *Tritylation*. The hydrochloride (5a) (2.04 g, 5 mmol) was dissolved in chloroform (10 ml) by the addition of triethylamine (0.7 ml, 5 mmol) at 0 °C. Trityl chloride (1.54 g, 5.5 mmol) and triethylamine (0.77 ml, 5.5 mmol) were added and the mixture was stirred for 1 h at room temperature and set aside overnight. The mixture was partitioned between ether and water, the layers were separated and the organic phase was washed quickly with 0.5N sulphuric acid, water, 2M potassium hydrogencarbonate solution, and again with water (to neutral pH), dried, and evaporated to dryness. The residual oil of N$^{\alpha}$-*benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-L-*lanthionine dimethyl ester* (yield quantitative) was homogeneous on t.l.c., R_F (1) 0.6.

(b) *Partial saponification*. To a solution of the above oily trityl derivative (20 mmol) in acetone (40 ml) potassium hydroxide (1N, 21 ml) was added dropwise with stirring, over a period of 20 min. After another 30 min, the solution was diluted with water (120 ml) and extracted twice with ether. The separated aqueous layer, after removing the organic solvent under reduced pressure, was acidified with acetic acid until it gave a slight Congo Red reaction, whereupon the amorphous *monoester* separated out. Water was decanted and the solid residue was triturated in a mortar with dilute acetic acid, yield 10.5 g (80%), m.p. 70—80 °C, R_F (1) 0.3. To remove inorganic salts this product was dissolved in ether–ethyl acetate (1 : 1) and the solution repeatedly washed with water. The organic layer was dried and evaporated to dryness. The residue was triturated with water, acidified with acetic acid, filtered off, and dried in high vacuum over phosphorus pentaoxide, m.p. 80 °C (not sharp); $[\alpha]_D^{27}$ +38.9° (*c* 2.5 in methanol) (Found: C, 68.2; H, 5.8; N, 4.6; S, 5.2; OMe, 5.1. $C_{34}H_{34}N_2O_6S$ requires C, 68.2; H, 5.7; N, 4.7; S, 5.35; OMe, 5.2%).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-(D→L)-*lanthionine* α′-*Methyl Ester* (6b).—Tritylation of the oily hydrochloride, described for (5b), was performed as under (6a) and gave a homogeneous oil (yield 88%), R_F (1) 0.6. Partial saponification was performed as described under (6a). The oily crude *product* was dissolved in a small quantity of warm ether and some undissolved material filtered off; the ethereal solution was added with stirring and cooling, in small portions to light petroleum, and each time the precipitated solid was collected by filtration; yield 81%, m.p. 60—75 °C, $[\alpha]_D^{25}$ +77.3° (*c* 2.5 in methanol), R_F (1) 0.3.

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*lanthionine* α′-*Methyl Ester Hydrochloride*.—Compound (6a) (4 g, 6.7 mmol) was dissolved in acetone (18 ml) and concentrated hydrochloric acid (0.69 ml) was added with stirring. After 2 min the *hydrochloride* was precipitated with ether; yield 2.2 g (84%), m.p. 148—150 °C after reprecipitation from methanol–ethyl acetate; $[\alpha]_D^{28}$ −19.4° (*c* 2 in methanol); paper electrophoresis (260 V, 2.5 h) in the system 1N-hydrochloric acid (50 ml)–1M-tris(hydroxymethyl)aminomethane (86.1 ml)–water (to 1 l) (pH 7.5), showed a single band which moved towards the cathode (Found: C, 45.3; H, 5.35; N, 6.9; S, 8.1; Cl, 9.2. $C_{15}H_{21}N_2O_6SCl$ requires C, 45.9; H, 5.4; N, 7.1; S, 8.2; Cl, 9.0%).

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*lanthionine* α-*Phenacyl* α′-*Methyl Diester Hydrochloride*.—(a) *Esterification*. The monoester (6a) (3 g, 5 mmol) was dissolved in ethyl acetate (12 ml); triethylamine (0.7 ml) and phenacyl bromide (1 g) were added at 0 °C and the solution was set aside overnight. The mixture was diluted with ethyl acetate, triethylamine salt was removed by filtration, and the filtrate was washed repeatedly with water, dried, and evaporated to give a homogeneous oil of N$^{\alpha}$-benzyloxycarbonyl-N$^{\alpha'}$-trityl α-phenacyl α′-methyl diester (yield quantitative); R_F (1) 0.6.

(b) *Detritylation*. To the above oily diester dissolved in a mixture of methanol (50 ml) and ethyl acetate (5 ml) hydrogen chloride in methanol (4.55N, 1.25 ml) was added and the solution was set aside overnight at room temperature. The solvent was evaporated off, the residue triturated with light petroleum, and the supernatant liquid decanted off. After addition of ethyl acetate and stirring the oily product solidified (1.45 g, 57%). It was recrystallized from isopropyl alcohol, m.p. 96—99 °C; $[\alpha]_D^{18}$ −19.7° (*c* 2 in methanol); R_F (1) 0.1 (Found: C, 54.0; H, 5.7; N, 5.15; S, 6.1; Cl, 6.4. $C_{23}H_{27}ClN_2O_7S$ requires C, 54.1; H, 5.3; N, 5.5; S, 6.3; Cl, 6.9%).

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*lanthionine* α-p-*Nitrophenyl* α′-*Methyl Diester Hydrochloride* (8a).—(a) *Esterification*. The monoester (6a) (7.5 g, 12.5 mmol) was dissolved in ethyl acetate (23 ml); the solution was cooled to 0 °C and *p*-nitrophenol (2.1 g) and dicyclohexylcarbodi-imide (2.9 g) were added. The mixture was stirred for 30 min at 0 °C, for 3 h at room temperature, and was then set aside overnight. Ether (150 ml) was added and the dicyclohexylurea was filtered off. Acetic acid (50%, 0.2 ml) was added to the filtrate and the solution was washed with cold water, 2M-sodium hydrogencarbonate solution, and again with water, and then dried and concentrated to give N$^{\alpha}$-*benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-L-*lanthionine* α-p-*nitrophenyl* α′-*methyl diester* (7a) as an oil (yield quantitative); R_F (1) 0.6.

(b) *Detritylation*. The above diester (7a) was dissolved in ether (20 ml) and hydrogen chloride in methanol (4.55N, 3 ml) was added. The hydrochloride (8a) separated out as an oil, which solidified after stirring for 3 h. It was set aside overnight at 4 °C, filtered off, triturated repeatedly with ether, and reprecipitated from dimethylformamide–ethyl acetate (5.2 g, 80%), m.p. 157—158 °C; $[\alpha]_D^{19}$ −25.7° (*c* 2 in dimethylformamide) (Found: C, 49.2; H, 4.7; N, 8.3; S, 6.2; Cl, 6.8. $C_{21}H_{24}ClN_3O_8S$ requires C, 49.1; H, 4.7; N, 8.2; S, 6.2; Cl, 6.9%).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\alpha'}$-*trityl*-(D→L)-*lanthionine* α-p-*Nitrophenyl* α′-*Methyl Diester* (7b).—This was prepared from the monoester (6b) as described for (7a). The oily diester was solidified from ethanol–water (1 : 1), m.p. 58—70 °C; $[\alpha]_D^{30}$ +76.5° (*c* 2.3 in ethyl acetate); R_F (6) 0.9; R_F (7) 0.9 (Found: C, 66.5; H, 5.3; N, 5.8; S, 4.5. $C_{40}H_{37}N_3O_8S$ requires C, 66.75; H, 5.2; N, 5.8; S, 4.45%).

N$^{\alpha}$-*Benzyloxycarbonyl*-(D→L)-*lanthionine* α-p-*Nitrophenyl* α′-*Methyl Diester Hydrochloride* (8b).—This was prepared from the N$^{\alpha}$-trityl diester (7b) as described for (8a). The crude product (yield 80%) was purified by reprecipitation (twice) from methanol–ether, m.p. 181—183 °C (sinters at 180 °C); $[\alpha]_D^{19}$ +36.2° (*c* 2 in dimethylformamide) (Found: C, 48.9; H, 4.6; N, 8.2; Cl, 7.1. $C_{21}H_{24}ClN_3SO_8$ requires C, 49.1; H, 4.7; N, 8.2; Cl, 6.9%).

N-*Benzyloxycarbonyl*-cyclo-L-*lanthionyl Methyl Ester* (2a).—(*a*) N$^{\alpha}$-Benzyloxycarbonyl-L-lanthionine α′-methyl ester hydrochloride (2.1 g, 5.35 mmol) was dissolved in dimethylformamide (430 ml). To the solution, cooled to −15 °C, were added successively a triethylamine solution (0.75 ml)

in dimethylformamide (54 ml), a solution of dicyclohexylcarbodi-imide (1.65 g) in dimethylformamide (54 ml), and *N*-hydroxysuccinimide (0.63 g); the mixture was stirred for 3 h at 0 °C, then set aside for 40 h at 4 °C and 5 d at room temperature, in the dark. The reaction was followed by t.l.c. and by optical rotation measurements. After 90 h, t.l.c. [system (1)] showed that the starting material (R_F 0.0) had disappeared, and an initial optical rotation of $[\alpha]_D^{22}$ −12.5° had changed to *ca.* 0°. Acetic acid was added to the solution and it was evaporated to dryness under high vacuum. To the solid residue, ethyl acetate was added and the dicyclohexylurea was filtered off. The filtrate was washed with 0.5N-sulphuric acid, potassium hydrogencarbonate solution, and water to neutrality, dried, and evaporated to dryness. The solid residue was triturated with cold ethyl acetate and filtered off to give the cyclo-*compound* (1.2 g, 67%), m.p. 167—168 °C after recrystallization from ethyl acetate; $[\alpha]_D^{20}$ +2.9° (*c* 2 in dimethylformamide), R_F (1) 0.55. A mixed melting point with a sample prepared as described before [1b] showed no depression.

(*b*) The hydrochloride (8a) (1 g, 2 mmol) was dissolved in dimethylformamide (100 ml), and *N*-hydroxybenzotriazole (0.27 g) followed by triethylamine (0.31 ml) were added. The solution was set aside at room temperature in the dark for 3 d during which time the reaction was followed by chromatography. The solvent was evaporated off under high vacuum at 35 °C, the solid residue was dissolved in ethyl acetate (100 ml) and the solution washed once with 4N-ammonium hydroxide, repeatedly with a saturated solution of sodium carbonate (to remove yellow impurities), and then with water to neutrality, dried, and evaporated to dryness, and the crystalline product was triturated with ether and filtered off (0.5 g, 75%), m.p. 168—169 °C; * $[\alpha]_D^{20}$ +3.5°, $[\alpha]_D^{30}$ +3.3 (*c* 5 in dimethylformamide),* R_F (1) 0.55 (Found: C, 53.2; H, 5.4; N, 8.1; S, 9.6. $C_{15}H_{18}N_2O_5S$ requires C, 53.2; H, 5.4; N, 8.3; S, 9.5%); for amino-acid analysis see Table 3.

N-*Benzyloxycarbonyl*-cyclo-(D→L)-*lanthionyl Methyl Ester* (2b).—This was prepared by the method (*b*) described for (2a), yield 75%. For recrystallization the product (240 mg) was dissolved in methanol (3 ml), undissolved material was removed by filtration, ether (20 ml) and light petroleum (2—3 ml) were added, and the mixture was set aside overnight at −10 °C. A first precipitate (5 mg) was filtered off; to the filtrate light petroleum (*ca.* 25 ml) was added; it was then set aside overnight at 4 °C and the cyclo-*compound* collected by filtration, m.p. 150—151 °C; $[\alpha]_D^{30}$ −2.6° (*c* 1 in dimethylformamide) (Found: C, 53.0; H, 5.4; N, 8.2; S, 9.5%); amino-acid analysis after hydrolysis with concentrated hydrochloric acid–acetic acid (1 : 1) for 5 or 15 h at 60 °C: lanthionine, 0%; *meso*-lanthionine, 100%; molecular-weight determination (osmometrically in dimethylformamide solution, at two concentrations): 331, 335 (calc. 338.4).

N-*Benzyloxycarbonyl*-cyclo-L-*lanthionine* (9).—The *cyclo*-ester (2a) (0.5 g) was dissolved in dioxan–water (15 ml, 1 : 1). To the cold (0 °C) solution 2N-sodium hydroxide (2.5 ml) was added dropwise and the solution stirred for 30 min at room temperature. Undissolved material was filtered off and the filtrate acidified with 1N-hydrochloric acid to precipitate the crystalline product (0.43 g, 90%), m.p. 174—177 °C, unchanged after recrystallization from methanol; for amino-acid analysis see Table 3 (Found: C, 49.1; H, 5.2; N, 8.1; S, 9.5; H_2O, 5.2. $C_{14}H_{16}N_2O_5S,H_2O$ requires C, 49.1; H, 5.3; N, 8.2; S, 9.4; H_2O, 5.3%).

cyclo-L-*Lanthionyl Hydrobromide* (10).—The *N*-protected compound (9) was dissolved in 3N-hydrogen bromide in acetic acid (3 ml), phosphorous acid diethyl ester (1 ml), and diethyl disulphide (1.5 ml); the solution was set aside for 40 min at room temperature and the mixture was poured into cold ether (50 ml); the precipitated oil was solidified by repeatedly decanting the supernatant liquid, adding new ether, and scratching. It was then dissolved in ethanol–water (12 ml, 1 : 1), the solution was evaporated, and the crystalline residue triturated with acetone and filtered off, yield 0.17 g (68%), m.p. 201—202 °C (decomp.) unchanged after recrystallization from water–acetone; R_F (3) 0.15, R_F (4) 0.5; for amino-acid analysis see Table 3 (Found: N, 10.1; S, 11.5; Br, 29.8. $C_6H_{11}BrN_2O_3S$ requires N, 10.3; S, 11.8; Br, 29.5%).

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* Unchanged after recrystallisation from ethyl acetate.

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