SYNTHESIS OF ANALOGS OF VALINOMYCIN AND ENNIATINE B CONTAINING CHARGED, SPIN-LABELED, OR FLUORESCENT GROUPS

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The synthesis has been performed of a large number of analogs of the membrane-active antibiotics valinomycin (1) and enniatine B (2); for many of them their capacity for forming complexes with sodium and potassium ions has been studied, their antimicrobial activity has been measured, and their conformational states have been investigated (see [1] and the literature cited therein). The results obtained include valuable information of the connection between structure and function in this series of cyclodepsipeptides, the function considered being the ionophoric activity of these compounds, i.e., their capacity for acting as carriers of ions through artificial and biological membranes. In this investigation, the role of such factors as the size of the ring, the configuration of the amino-acid and hydroxy-acid residues and the nature and size of the aliphatic side chains were studied.

The present communication gives the results of the synthesis of functional derivatives of valinomycin and enniatine B containing amine or carboxy groups in the side chains (compounds 3-6):

(1) [(-D-Val-L-Lac-L-Val-D-Hylv-)$_3$], Valinomycin

(2) [(-L-MeVal-D-Hylv-)$_3$] Ennniatine B

(3) [D-Val-L-Lac-L-Lys-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv)$_2$]

(4) [D-Val-L-Lac-L-Glu-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$],

(5) [L-MeLys-D-Hylv (-L-MeVal-D-Hylv-)$_2$],

(6) [L-MeGlu-D-Hylv (-L-MeVal-D-Hylv-)$_2$].

The interest in obtaining the compounds listed is due to the fact their side chains contain ionogenic groups which affect the total charge of the corresponding complex cations; in contrast to the singly positively-charged complexes (1)·M^+ and (2)·M^+, the complexes (3)·M^+ and (5)·M^+ may be doubly charged under certain conditions and (4)·M^+ and (6)·M^+ be electrically neutral (M represents an alkali metal). This fact, naturally, must be reflected in the ionophoric properties of the analogs 3-6. In particular, compounds 4 and 6 may be expected to have properties in common with the antibiotics of the nigericin group, a characteristic feature of which is the presence of carboxy groups and a capacity for forming electrically neutral complexes with metal ions [2-4]. A study of the dependence of the stability of complexes of compounds 3-6 on the pH of the medium will enable new information to be obtained on the nature of the forces responsible for complex formation, on the screening of the central ion in a complex, etc. Finally, the presence of reactive functional groups can be used for the covalent bonding of the molecules of the cyclodepsipeptides to various carriers, reagents, or spectral labels. As an example, on the basis of the lysine analogs 3 and 5, the spin-labeled derivatives 7 and 8 containing a stable iminoxyl radical, and also the corresponding dansyl derivatives 9 and 10 which it is proposed to use as fluorescence-labeled ionophores have been obtained.

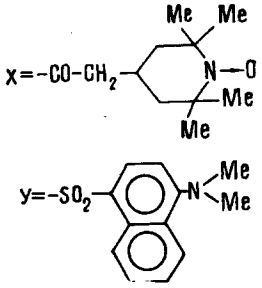
M. M. Shemyakin Institute of the Chemistry of Natural Compounds of the Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 346-358 May-June, 1974. Original article submitted February 12, 1973.

(7) [D-Val-L-Lac-L-Lys(X)-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$],

(8) [L-MeLys(X)-D-Hylv (-LMeVal-D-Hylv-)$_2$],

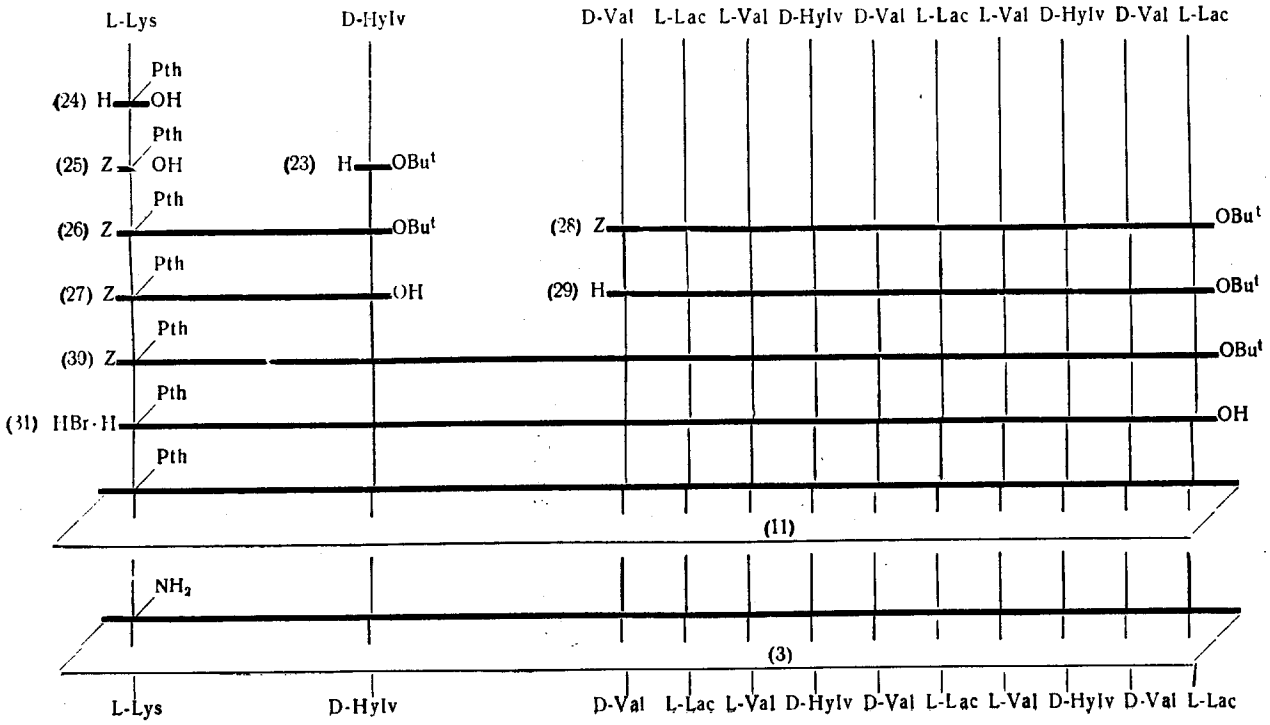
(9) [D-Val-L-Lac-L-Lys(Y)-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$],

(10) [L-MeLys(Y)-D-Hylv (-L-MeVal-D-Hylv)$_2$].

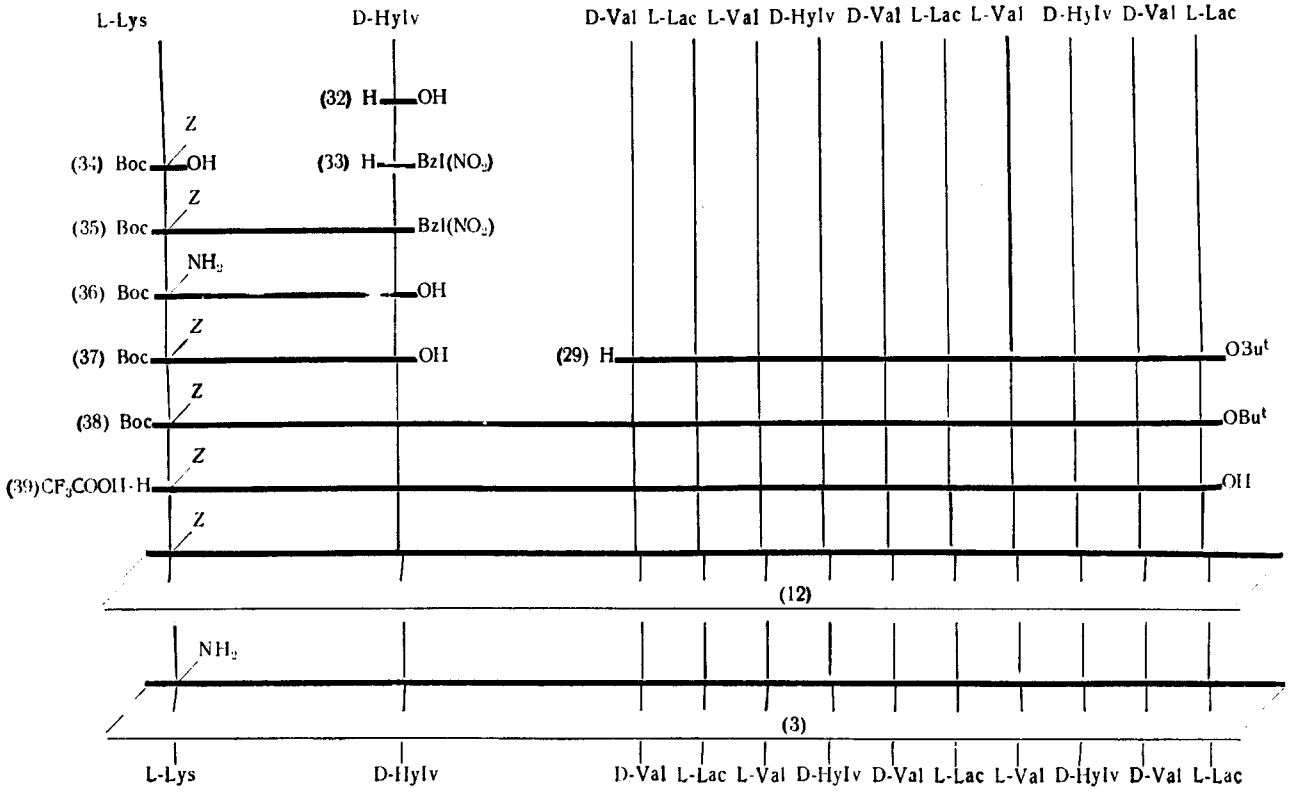


Compounds 3 and 4 were synthesized by Schemes 1-3 using methods developed previously [1] which ensure high yields and the optical purity of the depsipeptide. In the preparation of the linear depsipeptides, the ester bonds were created by the benzenesulfonyl chloride method, and the amide bonds by the acid chloride method; cyclization was also effected by the acid chloride method. The γ-carboxy function of the glutamic acid residue was protected by a p-nitrobenzyl group, which was removed after cyclization by hydrogenolysis. For protecting the ε-amino group of lysine, we first used the phthaloyl group, but its removal from the cyclodepsipeptide (11) was associated with considerable difficulty. Under mild conditions (one equivalent of hydrazine in ethanol, 30°C, 10 h), the phthaloyl group was removed from the cyclodepsipeptide (11) extremely slowly, and under more severe conditions (5 equivalents of hydrazine in ethanol, 80°C, 72 h) the hydrazinolysis of the ester bonds took place with the cleavage of the depsipeptide chain. The phthaloyl protection is removed considerably faster in the presence of potassium ions (apparently because of the peripheral arrangement of the side chain of the phthaloyllysine residue in the K^+ complex), but

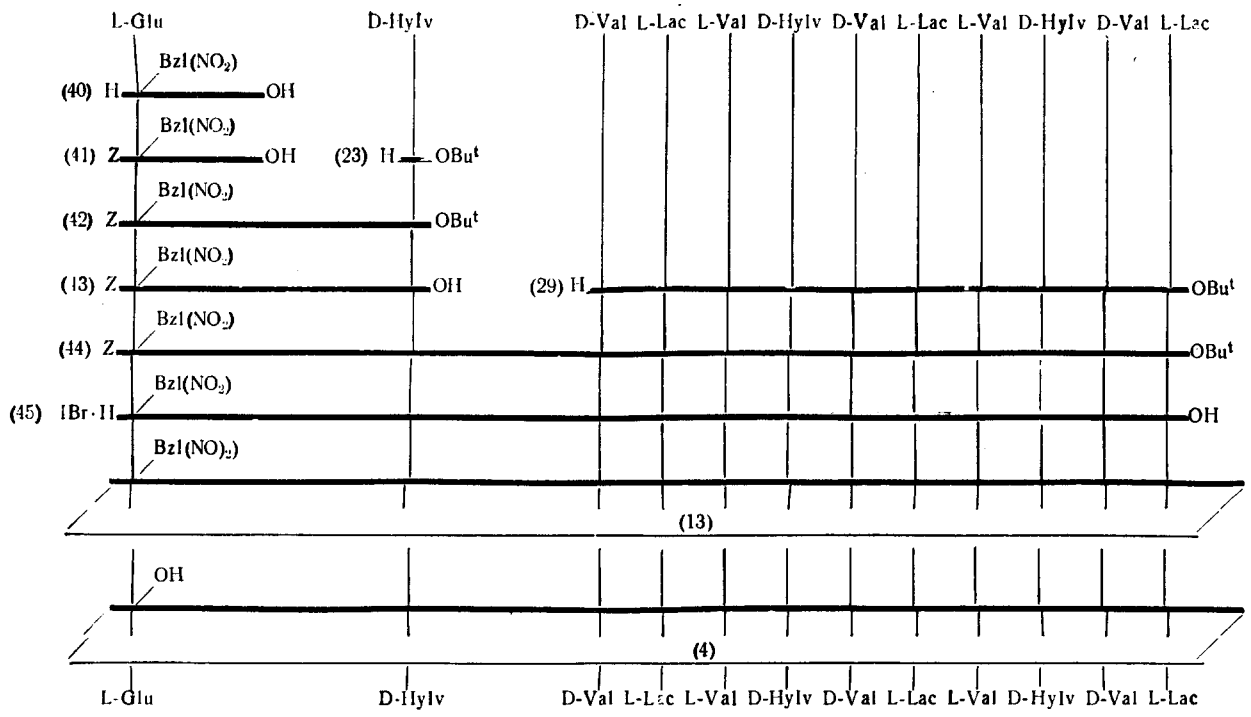
Scheme 1



Scheme 2



Scheme 3



in this case as well the yield of compound 3 did not exceed 30%. In view of this, we obtained the cyclodepsipeptide (12), the benzyloxycarbonyl group of which was easily removed by hydrogenolysis with quantitative yield.

Pth
(11) [D-Val-L-Lac-L-Lys-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$]

Z
(12) [D-Val-L-Lac-L-Lys-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$]

Bzl (NO_2)
(13) [D-Val-L-Lac-L-Glu-D-Hylv (-D-Val-L-Lac-L-Val-D-Hylv-)$_2$]

The functional derivatives of enniatine B (5 and 6) were obtained by eliminating the protective groups from compounds 13 and 14 isolated after the N-methylation of the cyclodepsipeptides (21 and 22). The latter, in their turn, were obtained by Schemes 4 and 5; the direct elimination of their protective groups led to the formation of the functional derivatives (17) and (18) of a biologically active analog of enniatine B – mono-N-demethylenniatine B (16) [5, 6].

Pth
(14) [L-MeLys-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

Bzl (NO_2)
(15) [L-MeGlu-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

(16) [L-Val-D-Hylv (-L-MeVal-D-Hylv-)$_2$] Mono-N-dimethylenniatine B

(17) [L-Lys-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

(18) [L-Glu-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

X
(19) [L-Lys-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

Y
(20) [L-Lys-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

Pth
(21) [L-Lys-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

Bzl (NO_2)
(22) [L-Glu-D-Hylv (-L-MeVal-D-Hylv-)$_2$]

The spin-labeled compounds (7), (8), and (19) were obtained from compounds (3), (5), and (17) and the N-hydroxysuccinimide ester of 1-oxyl-2,2,6-tetramethylpyridin-4-ylacetic acid; the reaction of compounds (3), (5), and (17) with dansyl chloride gave the fluorescence-labeled derivatives (9), (10), and (20).

The individuality of all the compounds was checked by thin-layer chromatography on silica gel. The structure of the cyclic depsipeptides was confirmed by the results of microanalysis and mass spectrometry. The investigation of the compounds obtained that is being performed at the present time by the methods of optical rotatory dispersion, circular dichroism, and IR, UV, and NMR spectroscopy also indicates their purity. The physicochemical constants of the cyclic products obtained and of the intermediates are given in Tables 1 and 2.

The antimicrobial activity of the cyclodepsipeptides synthesized was studied by the serial dilution method in relation of Gram-positive, Gram-negative, and acid-resistant bacteria, and also some yeast fungi (Table 3). This table also gives (for comparison) information on the antimicrobial activity of valinomycin, enniatine B, and mono-N-demethylenniatine B. It can be seen from these figures that the appearance of ionogenic groups sharply lowers the antimicrobial activity of the depsipeptide antibiotics, although in a number of cases (compounds 3 and 4) it does not lead to their complete inactivation. One of the possible causes of the reduction in activity is the lower lyophilicity of the molecular surface of complex cations of the type of cyclodepsipeptide $\cdot K^+$. So far as concerns the protected analogs, some of them (compounds 11 and 14) are not inferior in antimicrobial activity to the natural antibiotics while others (12, 13, and 22) are practically inactive. Further chemical and biophysical investigations are necessary to explain the reasons for such different behaviors of these compounds.

TABLE 1. Physicochemical Constants of the Cyclodepsipeptides*

Compound	$[\alpha]_D^{20}$, deg		Solvent	R_f	System	Mol. wt. (mass spectrometric)
(3)	+7,5	0,10	C_6H_6	0,8	1	1140
(4)	+15,0	0,10	C_6H_6	0,3	2	1155**
(5)	−52,5	0,20	C_6H_6	0,7	1	668 †
(6)	−74,0	0,20	C_6H_6	0,4	2	669
(7)	+4,0	0,10	C_2H_5OH	0,6	3	1336
(8)	+13,0	0,10	C_2H_5OH	0,55		864
(9)	+13,0	0,10	C_2H_5OH	0,65		1373
(10)	+32,0	0,14	$C_4H_8O_2$	0,6		901
(11)	+18,4	0,43	C_2H_5OH	0,7	4	1285
(12)	+32,0	0,09	C_6H_6	0,8		1274
(13)	+20,0	0,10	C_6H_6	0,7		1276
(14)	−44,1	0,20	C_6H_6	0,3		813
(15)	−69,8	0,20	C_6H_6	0,4		804
(17)	−77,8	0,20	C_6H_6	0,8	1	654
(18)	−10,0	0,20	C_6H_6	0,6	2	655
(19)	−74,9	0,10	C_2H_5OH	0,5	3	850
(20)	−38,0	0,10	C_2H_5OH	0,55		887
(21)	−8,2	0,20	C_6H_6	0,5	4	799
(22)	−91,1	0,20	C_6H_6	0,6		790

*All the compounds given in the table were obtained in the form of amorphous powders.
† The methyl ester of compound 4, obtained by treating it by diazomethane in ether, was used for the molecular weight determination.

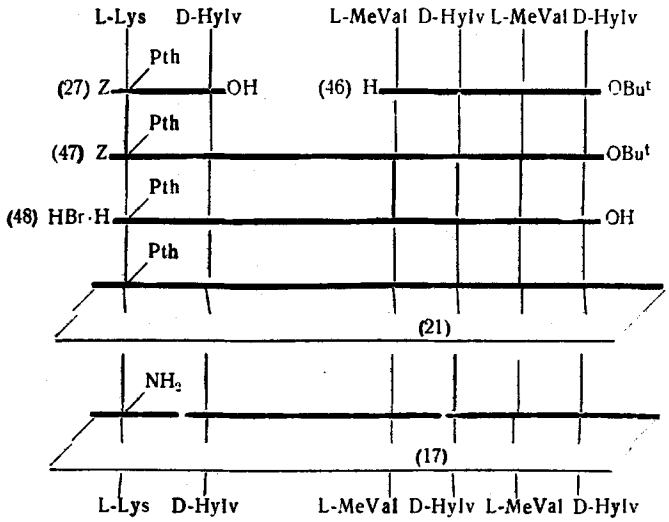
TABLE 2. Physicochemical Constants of the Intermediate Compounds

Compound	mp, °C	$[\alpha]_D^{20}$, deg		Solvent	R_f	System
(25)	120	−12,2	0,20	CH_3OH	0,8	2
(26)	Amorph.	−4,0	1,00	C_6H_6	0,6	5
(27)		−1,9	0,40	C_2H_5OH	0,5	3
(29)	Oil	−6,6	0,50	C_6H_6	0,35	4
(30)		−1,5	0,50	C_6H_6	0,5	
(33)	41-42	+5,9	0,40	C_2H_5OH	0,6	5
(35)	Oil	+8,4	0,40	C_6H_6	0,7	
(36)	220	−3,7	0,20	CH_3OH	0,2	1
(37)	Amorph.	+12,5	0,60	C_6H_6	0,5	3
(38)	Oil	+1,8	0,20	C_2H_5OH	0,5	
(41)	60	−15,5	0,20	CH_3COOH	0,7	5
(42)		−10,0	0,20	C_6H_6	0,5	
(43)		−7,0	0,27	C_6H_6	0,6	3
(44)	Oil	+2,8	0,30	C_2H_5OH	0,4	
(47)		−76,4	0,20	C_6H_6	0,6	5
(49)		−61,5	0,20	C_6H_6	0,7	

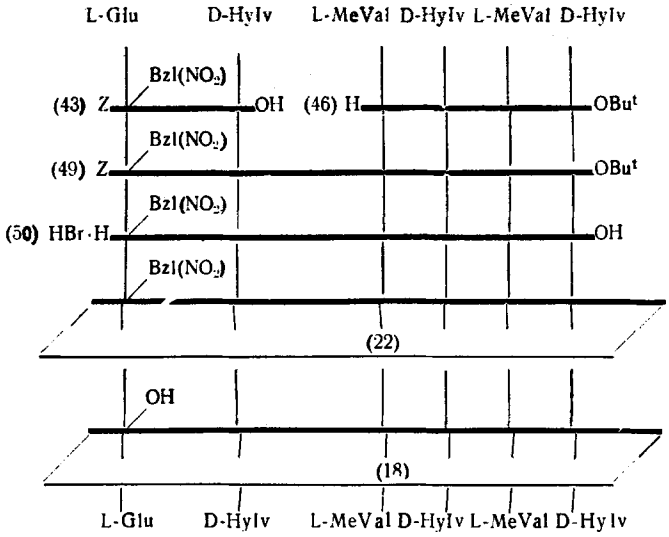
TABLE 3. Antimicrobial Activity of the Cyclodepsipeptides

Compound	Minimum concentration (γ/ml) suppressing the growth of								
	St. aureus 209-P	Sar. lutea	Str. faecalis	Bac. mycoides	Bac. subtilis	E. coli B	Mycob. phlei	Cand. albicans	Saccb. cerevisiae
(1)	10	0,1	0,2	10	10	10	0,3	0,2–0,4	0,2–0,4
(2)	18	9–12	18	25–37	37–50	50	9–12	12	12
(3)	25–37	25–37	>40	>40	>40	>40	25–37	>40	>40
(4)	>25	>25	>25	>25	>25	>25	6–9	>25	>25
(5)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(6)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(7)	>5	>5	>5	>5	>5	>5	>5	>5	>5
(8)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(9)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(10)	18	9–12	>40	>40	>40	>40	12	>40	>40
(11)	10	0,1–0,2	0,2	10	10	10	0,3	0,2–0,4	0,2–0,4
(12)	>25	>25	>25	>25	>25	>25	>25	>25	>25
(13)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(14)	7,5	7,5	12–18	>40	>40	>40	7,5–12	12–18	12–18
(15)	25–37	12–18	>40	>40	>40	>40	12–18	>40	>40
(16)	75	37–50	—	50–75	>100	>100	25–37	75	75
(17)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(18)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(19)	>80	>80	>80	>80	>80	>80	>80	>80	>80
(20)	>40	>40	>40	>40	>40	>40	>40	>40	>40
(21)	>40	12–18	>40	>40	>40	>40	12–18	>40	>40
(22)	>40	>40	>40	>40	>40	>40	>40	>40	>40

Scheme 4



Scheme 5



EXPERIMENTAL

All the melting points are uncorrected. The individuality of the compounds obtained was checked by thin-layer chromatography on silica gel ("Eastman" plates) in the following solvent systems: 1) butan-1-ol–pyridine–water–acetic acid (4:1:2:1); 2) dioxane–water (4:1); 3) chloroform–methanol (4:1); 4) benzene–ethyl acetate (1:2); and 5) benzene–ethyl acetate (2:1). For all the compounds the results of elementary analysis agreed satisfactorily with the calculated figures for C, H, and N.

1. N-Benzyloxycarbonyl-γ-p-nitrobenzyl Ester of L-Glutamic Acid (41) and Benzyloxycarbonyl-ε-N-phthaloyl-L-lysine (25). The γ-p-nitrobenzyl ester of L-glutamic acid (40) [7] (0.01 mole) or ε-N-phthalolyl-L-lysine (24) [8] (0.01 mole) and $NaHCO_3$ (2 g; 0.024 mole) were suspended in 180 ml of a mixture of dioxane and water (4:1), and 2 ml (0.012 mole) of benzyl chloroformate was added at 20°C, after which the mixture was stirred for 20 h. Then it was extracted with ether, made alkaline with HCl, and extracted with ethyl acetate; extract was dried with $MgSO_4$ and evaporated. The residue was recrystallized from a mixture of ether and hexane [to isolate compound (41)] or from methylene chloride [for the isolation compound (25)]. Yields 80%.

2. p-Nitrobenzyl Ester of D-α-Hydroxyisovaleric Acid (33). A mixture of 11.8 g (0.1 mole) of D-α-hydroxyisovaleric acid (32), 21.6 g (0.1 mole) of p-nitrobenzyl bromide, and 13.9 ml (0.1 mole) of tri-

ethylamine in 120 ml of ethyl acetate was boiled for 7 h. The precipitate of triethylamine hydrobromide was filtered off, 6 ml of methanol was added to the filtrate, the solution was cooled, and it was washed successively with water, 1 N HCl, saturated in $NaHCO_3$ solution and water, dried with $MgSO_4$, and evaporated in vacuum. The residue was recrystallized from a mixture of ethyl acetate and hexane. Yield 21 g (83%).

3. The Didepsipeptides (26, 35, and 42). With stirring, (0°C, 10 min) 0.11 mole of freshly distilled benzenesulfonyl chloride was added to a solution of 0.12 mole of a N-protected amino acid with a blocked side group (25, 34 [9], or 41) in 100 ml of dry pyridine. After 15 min at 0°C, 0.1 mole of the tert-butyl or p-nitrobenzyl ester of D-α-hydroxyisovaleric acid (23 [10] or 32) in 20 ml of dry pyridine was added. The reaction mixture was stirred at 0°C for 2 h and then at 20°C for 3 h, poured into water, and extracted with ether. The extract was washed with a 1 N solution of H_2SO_4, a saturated solution of $NaHCO_3$, and water, dried with $MgSO_4$, and evaporated. The oil obtained was chromatographed on neutral alumina in the hexane–benzene–ethyl acetate system (gradient elution). Yield 75-85%.

4. α-tert-Butoxycarbonyl-L-lysyl-D-α-hydroxyisovaleric Acid (36). A mixture of 6.1 g (0.01 mole) of p-nitrobenzyl α-tert-butoxycarbonyl-ε-benzyloxycarbonyl-L-lysyl-D-α-hydroxyisovaleric acid (35) and 1.2 g (0.02 mole) of CH_3COOH in 60 ml of absolute dioxane was hydrogenated in the presence of a palladium catalyst with the passage of a current of hydrogen through the solution for 12 h. The catalyst was filtered off, the methanol was evaporated off, and the resulting powder was washed with dry ether and recrystallized from methanol. Yield 3.3 g (83%).

5. α-tert-Butoxycarbonyl-ε-benzyloxycarbonyl-L-lysyl-D-α-hydroxyisovaleric Acid (37). With vigorous stirring, (0°C, 15 min), 2 ml (0.02 mole) of benzyl chloroformate were added dropwise to 34 g (0.01 mole) of α-tert-butoxycarbonyl-L-lysyl-D-α-hydroxyisovaleric acid (36) and 4.2 g (0.05 mole of $NaHCO_3$ in 70 ml of water and 10 ml of dioxane). The mixture was stirred at 20°C at pH 8 for 48 h. Then it was extracted with ether, and the bicarbonate fraction was acidified with 10% citric acid and extracted with ethyl acetate, after which the ethyl acetate extract was dried with $MgSO_4$ and evaporated. The resulting oil was chromatographed on silica gel in the benzene–ethyl acetate system (gradient elution). Yield 3.5 g (73%).

6. The Acids (27 and 43). A protected depsipeptide (26 or 42) (50 mmole) was dissolved in 15 ml of trichloroacetic acid and the solution was kept at 20°C for 40-50 min. The excess of acid was evaporated off in vacuum at a bath temperature not exceeding 50°C, the residual oil was dissolved in 10 ml of dry toluene and the solvent was again distilled off in vacuum. The residue was dissolved in ether and extracted with a saturated solution of $NaHCO_3$; the bicarbonate extract was acidified with concentrated HCl and extracted with ether, and the ethereal extract was dried with $MgSO_4$ and evaporated. Yield 85-95%.

7. The Protected Hexapeptides (47 and 49). A solution of 10 mmole of the protected depsipeptide (27 or 43) in 35 ml of thionyl chloride was kept at 20°C for 1 h. The thionyl chloride was distilled off in vacuum at a bath temperature not exceeding 30°C, and then absolute benzene was added to the residue and this was distilled off in vacuum. The acid chloride obtained was dissolved in 50 ml of absolute ether and added dropwise (−30°C, 1 h), to a solution of 10 mmole of the amino ester (46) [10] and 1 ml (15 mmole) of triethylamine in 50 ml of absolute ether; and then the mixture was stirred at −30°C for 1 h and at 20°C for 2 h. The reaction mixture was washed with 1 N H_2SO_4 and with a saturated solution of $NaHCO_3$, dried with $MgSO_4$, and evaporated. The residue was chromatographed on neutral almina in the benzene–ethyl acetate system (gradient elution). Yield 85%.

8. The Decadepsipeptide tert-Butyl Ester (29). The hydrogenation of 22.4 g (20 mmole) of the tert-butyl ester of the protected decadepsipeptide (28) [11] was performed in solution in 50 ml of methanol in the presence of a palladium catalyst with the passage of a current hydrogen through the solution for 4 h. Then the catalyst was filtered off, and the solvent was distilled off in vacuum, giving 15.7 g (80%) of the amino ester (29).

9. The protected dodecadepsipeptides (30, 38, and 44) were obtained in the form of amorphous powders from the protected acids (27, 37, and 43) and the decadepsipeptide tert-butyl ester (29) under the conditions of experiment 6 using benzene instead of ether (0-5°C). Yield 85-95%.

10. Cyclization of the Linear Depsipeptides. A. A solution of 10 mmole of a protected depsipeptide (30, 44, 47, or 49) in 10 ml of glacial acetic acid was treated with 60 ml of a 35% solution of HBr in glacial acetic acid. The mixture was kept at 20°C for 1 h, and the solvent was carefully distilled off in vacuum, giving the corresponding hydrobromide (31, 45, 48, or 50) in the form of an oil.

B. In the case of the depsipeptide (38), 10 mmole was dissolved in 30 ml of aqueous trifluoroacetic

acid (15% of water) and the solution was kept at 20°C for 40 min, after which the solvent was carefully distilled off in vacuum, giving the trifluoroacetate (39).

C. Without additional purification, 3 mmole of a depsipeptide hydrobromide (31, 45, 48, or 50) or the depsipeptide trifluoroacetate (39) was dissolved in 30 ml of freshly distilled thionyl chloride, the solution was kept at 20°C for 1 h, the excess of thionyl chloride was carefully distilled off in vacuum, the residue was treated with 20 ml of absolute benzene, and the resulting solution was evaporated in vacuum. The residual hydrochloride was dissolved in 800 ml of absolute benzene and with stirring (20°C, 12 h) this solution was gradually added simultaneously with a solution of 30 mmole of triethylamine in 800 ml of absolute benzene to 2.5 liters of absolute benzene. Then the mixture was left for 20 h and was concentrated to a volume of 500 ml. It was washed with 5% HCl, water, and saturated $NaHCO_3$ solution, dried with $MgSO_4$, and evaporated. Compounds 11, 12, and 13 were isolated by chromatography on a column of neutral alumina (activity grade III) with gradient elution in the benzene–ethyl acetate system with a yield of 20-30%. Compounds 21 and 22 were dissolved in ethyl acetate and a saturated solution of KCNS was added until the precipitation of the complex ceased. The complex was filtered off and recrystallized from methanol. To decompose it, a suspension in ethyl acetate was shaken with water, saturated with $MgSO_4$ and was then evaporated. Yield 40%.

11. Methylation of the Cyclic Hexadepsipeptides (21 and 22). A mixture of 0.34 mmole of a cyclohexadepsipeptide (21) or (22) in 20 ml of absolute dimethylformamide, 4.0 g of silver oxide, and 15 ml of methyl iodide was shaken in a sealed tube at 20°C for 96 h. The precipitate was filtered off and was washed on the filter with 15 ml of dimethylformamide, the combined filtrates were evaporated in vacuum to a volume of 3 ml, 50 ml of chloroform were added, and the resulting solution was washed with 5% KCN solution (2× 10 ml), dried with $MgSO_4$, and evaporated. The residue was chromatographed on neutral alumina in the benzene–ethyl acetate system (gradient elution), and compounds (14) and (15) were isolated with yields of 20-30%.

12. The Cyclodepsipeptides (4, 6, and 18). A cyclodepsipeptide p-nitrobenzyl ester (13, 15, or 22) (0.1 mmole) and acetic acid (0.12 mmole) were dissolved in 20 ml of absolute dioxane and hydrogenation was performed in the presence of a palladium catalyst with the passage of a current of hydrogen for 10 h. The catalyst was filtered off and the solution was diluted with water (15%), and, to eliminate p-toluidine, it was passed through the ion-exchange resin Dowex 50× 2 in the H^+ form. The filtrate was evaporated giving the acid (4, 6, or 18) with a yield of 85-90%.

13. The Cyclodepsipeptides (3, 5, and 17). A. A mixture of 0.1 mmole of a phthaloylcyclodepsipeptide (14 or 21) in 2 ml of ethanol and 0.1 ml (0.1 mmole) of a 1 N ethanolic solution of hydrazine was boiled for 1 h and was then left at −5°C for 24 h. The precipitate that had deposited was filtered off, the solution was diluted with water (15%) and, to eliminate the phthaloylhydrazine it was passed through the ion-exchange resin Dowex 1× 4 in the HCO_3^- form. The filtrate was passed through the resin Dowex 50× 2 in the H^+ form, after which the required product was eluted with a 10% solution of triethylamine in aqueous ethanol (15% of water). After evaporation of the filtrate, the yield was 60%.

B. The potassium complex of the phthaloyl depsipeptide (11) (obtained under the conditions of experiment 10C) (0.1 mmole) was boiled with 0.3 mmole of hydrazine in 3 ml of ethanol for 20 h. After working up as in paragraph A, the yield was 30%.

C. A solution of 0.1 mmole of the benzyloxycarbonyldepsipeptide (12) in 20 ml of methanol was hydrogenated in the presence of a palladium catalyst with the passage of a current of hydrogen for 3 h. Then the catalyst was filtered off, giving substance 3 with a yield of 98%.

14. The Spin-Labeled Compounds (7, 8, and 19). A mixture of 0.1 μmole of compounds (3, 5, or 17) and 0.13 μmole of the N-hydroxysuccinimide ester of 1-oxyl-2,2,6,6-tetramethylpyridin-4-ylacetic acid (obtained from the corresponding acid [12] and N-hydroxysuccinimide by the carbodiimide method [13]) was dissolved in 1 ml of absolute dimethylformamide and the solution was stirred at 20°C for 24 h. Then the solvent was evaporated and the residue was dissolved in methanol and chromatographed on a column of Sephadex LH-20 (200× 0.8 cm). The high-molecular-weight fraction gave compounds (7, 8, or 19, respectively) with a yield of 70%.

15. The Dansyl Derivatives (9, 10, and 20). A solution of 0.1 μmole of a compound (3, 5, or 17) in 1 ml of a saturated solution of $NaHCO_3$ was treated with a solution of 0.13 μmole of dansyl chloride in 1 ml of acetone, and the mixture was stirred at 20°C for 24 h. Then the solvent was evaporated off and the residue was dissolved in ethyl acetate, and this solution was washed with water, with saturated $NaHCO_3$ solution, and again with water, dried with $MgSO_4$, and evaporated. The residue was dissolved in methanol and chromatographed on a column of Sephadex LH-20 (200× 0.3 cm). The high-molecular-weight fraction gave

compounds (9, 10, or 20, respectively) with a yield of 70%. The 1-oxyl-2,2,6,6-tetramethylpyridin-4-ylacetic acid was given to us by A. B. Shapiro.

CONCLUSIONS

1. The synthesis of six functional derivatives of valinomycin and enniatine B containing amine or carboxy groups in the side chain has been effected.

2. Four analogs of valinomycin and enniatine B containing spin-labeled or fluorescent groups have been obtained.

3. The antimicrobial activity of the compounds mentioned and also of the intermediate cyclodepsipeptides obtained during their synthesis has been studied.

LITERATURE CITED

1. Yu. A. Ovchinnikov, XXIII-rd International Congress of Pure and Applied Chemistry, Vol. 2, Butterworths, London (1971), p. 121.
2. W. K. Lutz, H. K. Wipf, and W. Simon, Helv. Chim. Acta, 53, 1741 (1970).
3. M. Pinkerton and L. K. Steinrauf, J. Mol. Biol., 49, 533 (1970).
4. M. Shiro and N. Koyama, J. Chem. Ser. B, 243 (1970).
5. R. O. Studer, P. Quitt, E. Bohni, and K. Vogler, Monatsh., 96, 461 (1965).
6. I. I. Mikheleva, I. D. Ryabova, T.A. Romanova, T.I. Tarasova, V. T. Ivanov, Yu. A. Ovchinnikov, and M. M. Shemyakin, Zh. Obshch. Khim., 38, 1228 (1968).
7. R. Ledger and F.H. C. Stewart, Austr. J. Chem., 18, 1477 (1965).
8. G. H. L. Nefkens, G. J. Tesser, J. Am. Chem. Soc., 83, 1263 (1961).
9. G. W. Anderson and A. C. McGregor, J. Am. Chem. Soc., 79, 6180 (1957).
10. A. Plattner, K. Vogler, R. O. Studer, P. Quitt, and W. Keller-Scierlein, Helv. Chim. Acta., 46, 927 (1969).
11. L. A. Fonina, A. A. Sanasaryan, and E. I. Vinogradova, Khim. Prirodn. Soedin., 69 (1971).
12. A. B. Shapiro, K. Baimagambekov, M. G. Gol'dfel'd, and É. G. Rozantsev, Zh. Organ. Khim., 11, 2263 (1972).
13. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, J. Am. Chem. Soc., 85, 3039 (1963).