Z-Tyr(Z)-NHNMe₂. The procedure for Z-Phe-NHNMe₂ was followed using 18.0 g (0.040 mol) of Z-Tyr(Z). The crude product was washed with cyclohexane to yield 17.6 g (90%), mp 152–159°. Crystallization from *i*-PrOH gave small needles, mp 160–163°, $[\alpha]^{28}D$ +4° (*c* 1, MeOH). *Anal.* Calcd for $C_{27}H_{29}N_3O_6$: C, 65.97; H, 5.95; N, 8.55. Found: C, 66.22; H, 6.06; N, 8.57.

Dipeptides. Coupling reactions were carried out by the *p*-nitrophenyl ester method.[12] When dimethylformamide was the solvent, the amino ester salt could be used directly followed by 1 equiv of a tertiary amine. In other cases, it was necessary first to liberate the amino ester and isolate it. The peptide reaction mixture was usually diluted with ethyl acetate and washed with dilute hydrochloric acid and thoroughly with potassium carbonate solution. The desired product was readily separated from unreacted active ester by crystallization.

Hydrogenations were done in 75 or 90% acetic acid over 5–10% by weight of palladium black at room temperature and up to 4 atm pressure. No difficulties were experienced even with S-methylcysteine and methionine at these high catalyst ratios.

Solid-Phase Synthesis of the Cyclododecadepsipeptide Valinomycin[1]

B. F. Gisin, R. B. Merrifield, and D. C. Tosteson

Contribution from The Rockefeller University, New York, New York 10021, and the Department of Physiology, Duke University, Medical Center, Durham, North Carolina. *Received October 4, 1968*

Abstract: The method of automated solid-phase peptide synthesis was applied to the preparation of the antibiotic valinomycin, a cyclic dodecadepsipeptide containing D-valyl, L-valyl, D-α-hydroxyisovaleryl, and L-lactyl residues. The open-chain depsipeptide was synthesized by coupling alternately the N-Boc-protected didepsipeptides L-valyl-D-α-hydroxyisovaleric acid and D-valyl-L-lactic acid to resin-bound D-valyl-L-lactate using dicyclohexylcarbodiimide as coupling agent. After cleavage from the resin the peptide was cyclized by the acid chloride method to give valinomycin. The crystalline product that was obtained in an over-all yield of 33% had the same physical and chemical properties as the natural antibiotic and showed the same characteristics in making lipid bilayers selectively permeable to potassium ions.

The antibiotic valinomycin was isolated from *Streptomyces fulvissimus* by Brockmann[2] in 1955, and a cyclooctadepsipeptide[3] structure was proposed for it.[4] The correct structure was finally established when Shemyakin, *et al.*,[5] synthesized the cyclododecadepsipeptide of the formula in Figure 1 and showed it to be identical with natural valinomycin. It contains two amino acids (L-valine and D-valine) and two hydroxy acids (D-α-hydroxyisovaleric acid and L-lactic acid),[6] which are arranged in a 36-membered ring regularly alternating between amino and hydroxy acids.

In recent years valinomycin has attracted the attention of several groups of investigators because of its remarkable effect on the permeability of biological and artificial lipid membranes to monovalent cations. The compound produces marked selectivity for K^+, compared with Na^+, in membranes of mitochondria,[7] in red blood cells,[8] and in several types of lipid bilayers.[9-12] Furthermore, under nonaqueous conditions this depsipeptide forms complexes much more readily with K^+ than with Na^+,[13,14] a property which is undoubtedly related to its effects on membranes.

The present investigation was undertaken in order to acquire more information about the relationship between the primary structure of the depsipeptide and its effects on the permeability of membranes to monovalent cations. Since a rapid way to prepare analogs of valinomycin was required, a method involving the principles of solid-phase peptide synthesis[15,16] was adapted to the synthesis of this depsipeptide.

Solid-phase peptide synthesis was first used to make a depsipeptide by Semkin, Smirnova, and Shchukina[17] who prepared an angiotensin analog containing one hydroxy

(1) Supported by Contract 14-01-001-1309, Office of Saline Water, U. S. Department of Interior, and in part by U. S. Public Health Service Grant AM 1260.

(2) H. Brockmann and G. Schmidt-Kastner, *Chem. Ber.*, **88**, 57 (1955).

(3) For a review article on cyclodepsipeptides, see D. W. Russell, *Quart. Rev.* (London), **20**, 559 (1966).

(4) H. Brockmann and H. Geeren, *Ann.*, **603**, 217 (1957).

(5) M. M. Shemyakin, N. A. Aldanova, E. I. Vinogradova, and M. Yu. Feigina, *Tetrahedron Letters*, **28**, 1921 (1963). Shemyakin and coworkers have also prepared a large number of compounds related to valinomycin and have tested them for antimicrobial and ion transport inducing properties: M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, N. F. Loginova, I. D. Ryabova, and I. A. Pavlenko, *Experientia*, **21**, 548 (1965).

(6) The abbreviations recommended by the IUPAC-IUB commission on Biochemical Nomenclature (*J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967)) have been used throughout. In addition: Hyv = α-hydroxyisovaleric acid and Lac = lactic acid.

(7) C. Moore and B. C. Pressmann, *Biochem. Biophys. Res. Commun.*, **15**, 562 (1964).

(8) D. C. Tosteson, P. Cook, T. Andreoli, and M. Tieffenberg, *J. Gen. Physiol.*, **50**, 2513 (1967).

(9) A. A. Lev and E. P. Buzhinsky, *Tsitologiya*, **9**, 102 (1967).

(10) P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967).

(11) T. E. Andreoli, M. Tieffenberg, and D. C. Tosteson, *J. Gen. Physiol.*, **50**, 2527 (1967).

(12) D. C. Tosteson, T. E. Andreoli, M. Tieffenberg, and P. Cook, *ibid.*, **51**, 373 (1968).

(13) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, A. M. Shkrob, I. I. Mikhaleva, A. V. Estratov, and G. G. Malenkov, *Biochem. Biophys. Res. Commun.*, **29**, 834 (1967).

(14) H. K. Wipf, L. A. R. Pioda, Z. Stefanac, and W. Simon, *Helv. Chim. Acta*, **51**, 377 (1968).

(15) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).

(16) R. B. Merrifield, *ibid.*, **86**, 304 (1964); *Science*, **150**, 178 (1965); *Recent Progr. Hormone Res.*, **23**, 451 (1967); *Advan. Enzymol.*, in press.

(17) E. P. Semkin, A. P. Smirnova, and L. A. Shchukina, *Zh. Obshch. Khim.*, **37**, 1169 (1967).

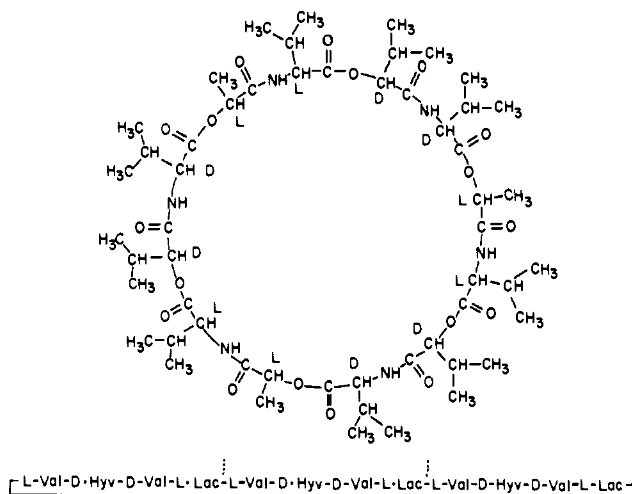

Figure 1. Valinomycin.

acid. They have also synthesized a tetradepsipeptide containing three α-hydroxy acids.[18] In each instance the ester bond was formed in pyridine by benzensulfonyl chloride activation, with a condensation time of 1 or 2 days. The formation of ester bonds requires much stronger activation than for peptide bonds and is therefore far less satisfactory.

The highly regular and repetitive structure of valinomycin suggested a different strategy for its synthesis. Instead of coupling the residues stepwise in 11 alternating amide- and ester-forming steps to a resin-bound residue the task was reduced to five peptide bond-forming steps by preforming all ester bonds in solution. Therefore, in the stepwise synthesis units of the type, $H_2NCH(R)COOCH(R')COOH$, were used. These didepsipeptides with an amino end and a carboxyl end could be substituted for α-amino acids in the standard procedure of solid-phase peptide synthesis. In contrast to dipeptides, racemization could not be facilitated by the formation of an oxazolone intermediate during activation of the carboxyl group.

The two N-protected didepsipeptides, *t*-butyloxycarbonyl-L-valyl-D-α-hydroxyisovaleric acid (I) and *t*-butyloxycarbonyl-D-valyl-L-lactic acid (II), representing fragments of valinomycin were prepared as outlined in Figure 2. The carboxyl groups of the α-hydroxy acids, D-α-hydroxyisovaleric acid, and L-lactic acid, were protected by esterification in benzyl alcohol with HCl. Boc-valine (L and D, respectively) was coupled to the free hydroxyl with N,N'-carbonyldiimidazole (CDI).[19] Debenzylation by hydrogenolysis gave Boc-D-Val-L-Lac-OH (I) and Boc-L-Val-D-Hyv-OH (II). The yield by the CDI method was superior to that by the mixed-anhydride method with benzene sulfonyl chloride, which is frequently applied to similar syntheses.[5,20-22]

For the ultimate cyclization step it was desirable to minimize interactions of bulky side chains. Accordingly, D-Val-L-Lac was chosen as the C-terminal fragment,

(18) L. A. Shchukina, E. P. Semkin, and A. P. Smirnova, *Khim. Prir. Soedin.*, **3**, 358 (1967); *Chem. Abstr.*, **68**, 87567*r* (1968).
(19) H. A. Staab, *Angew. Chem.*, **71**, 194 (1959).
(20) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, **19**, 581 (1963).
(21) P. Quitt, R. O. Studer, and K. Vogler, *Helv. Chim. Acta*, **47**, 166 (1964).
(22) G. Losse and H. Raue, *Chem. Ber.*, **101**, 1532 (1968).

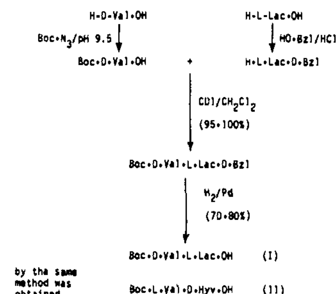

Figure 2. Synthesis of fragments of valinomycin.

rather than L-Val-D-Hyv. It was bound as a N-Boc derivative to a chloromethylated styrene–divinylbenzene resin in the usual way.[16,23] For the remainder of the synthesis the resin (III) was transferred to an instrument for the automated synthesis of peptides.[24] The machine was programmed and equipped to perform automatically the sequence of operations necessary to lengthen the peptide chain by one didepsipeptide unit: deprotection by HCl–dioxane, neutralization by Et_3N, choice of the proper didepsipeptide unit, I or II, addition of coupling agent N,N'-dicyclohexylcarbodiimide, and thorough washing with appropriate solvents in between. The cycle was repeated five times to give, on the resin, the protected linear dodecadepsipeptide with the sequence of valinomycin (V) according to Figure 3.

After each coupling step a sample of the peptide-resin was cleaved, and the liberated depsipeptide was subjected to tlc (Table I). Except at the level of the tetradepsipeptide (IV) where a small amount of D-Val-L-Lac was found, the intermediates proved to be practically homogeneous. At the tetradepsipeptide-resin stage ir spectroscopy was useful to show that coupling had occurred.

Since valine is the only amino acid present in valinomycin, quantitative amino acid analysis did not promise to be a very precise way to follow the synthesis. Nevertheless, the increase in valine content of the resin at each stage was observed to be between 80 and 110% of the calculated amount.

The dodecadepsipeptide was cleaved from the resin with hydrogen bromide in trifluoroacetic acid. The procedure also removed the Boc group but did not damage the ester bonds within the chain. The product was dissolved in acetic acid and precipitated in water to give H-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-OH·HOAc in 64% yield. The linear depsipeptide was homogeneous by tlc in three systems, each of which could separate it from lower homologs of the series (Table I).

In the first synthesis of valinomycin Shemyakin,[5] *et al.*, converted H-[D-Val-L-Lac-L-Val-D-Hyv]$_3$-OH into the acid chloride with thionyl chloride and cyclized at high dilution under basic conditions to yield 10% of the macrocyclic depsipeptide. In the present experiments the same cyclization method was used but the linear depsipeptide

(23) For a comprehensive review on solid-phase peptide synthesis, see J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman and Co., San Francisco, Calif., 1969.
(24) R. B. Merrifield, J. M. Stewart, and N. Jernberg, *Anal. Chem.*, **38**, 1905 (1966).

Table I. R_f Values of Depsipeptides on Silica Gel Tlc Plates

Depsipeptide[a] (as hydrobromide)	R_f[b] A	B	C
H-D-Val-L-Lac-OH	0.50	0.55	
H-L-Val-D-Hyv-OH	0.60	0.59	
H-L-Val-D-Hyv-D-Val-L-Lac-OH[c]	0.63	0.69	
H-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-OH	0.65	0.70	
H-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-OH	0.68	0.72	0.78
H-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-OH	0.69	0.73	0.76
H-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-L-Val-D-Hyv-D-Val-L-Lac-OH	0.71	0.76	0.81
Valinomycin[d]	0.88	0.94	0.88

[a] The crude cleaved intermediates contained only traces of impurities (by ninhydrin and iodine–tolidine reaction), with one exception (footnote *c*). [b] Solvent systems: A, 1-propanol–water (7:3, v/v); B, 1-butanol–acetic acid–pyridine–water (15:10:3:2, v/v); and C, 1-butanol–acetic acid–water (4:1:1, v/v). [c] The tetradepsipeptide was contaminated by approximately 5% of H-D-Val-L-Lac-OH. [d] Chloroform–methanol (2:1, v/v), R_f 0.85; 1-propanol–water (1:1, v/v), R_f 0.80.

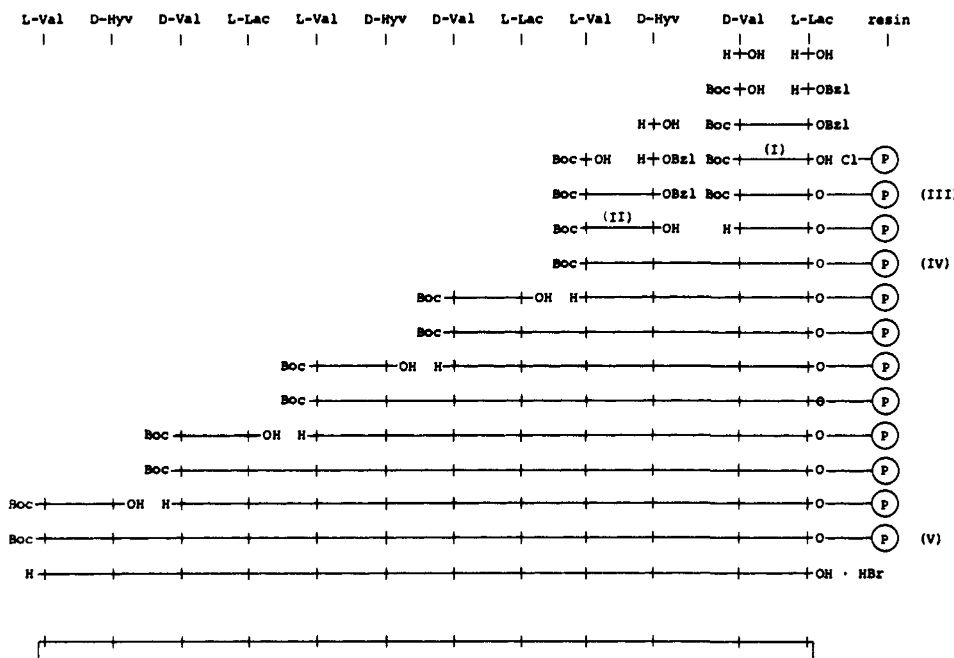

Figure 3. Scheme of the solid-phase synthesis of valinomycin.

was H-[L-Val-D-Hyv-D-Val-L-Lac]$_3$-OH, thus forming a L-Lac–L-Val bond instead of the more hindered D-Hyv–D-Val bond. In this case the yield of the cyclization was 51%. The product was purified by washing, and finally by recrystallization from dibutyl ether or nitromethane.

The purity and identity of the synthetic valinomycin was established in several ways. It was homogeneous by tlc (Table I). The ir spectrum was identical with a sample of natural valinomycin and with the spectrum published by Brockmann and Schmidt-Kastner.[2] There was no mixture melting point depression with the natural antibiotic.[25] The specific optical rotation was within experimental error of the value for valinomycin.[2] Quantitative amino acid analyses and elemental analyses were in agreement with the formula in Figure 1. Finally, the product was tested for its influence on the electrochemical properties of a lipid bilayer (Table II). Measurements were made both of the dc electrical resistance and the biionic potential of thin membranes prepared from sheep erythrocyte lipids.[11,12]

With 0.1 *M* KCl on both sides of the membrane, the resistance decreased approximately 40,000-fold when either synthetic or natural valinomycin was added in a concentration of 10^{-7} *M*. These results compare well with the data of Mueller and Rudin[10] who found that natural valinomycin at ten times the concentration caused a ten times greater decrease in membrane resistance. The biionic potential measurements were made with 0.1 *M* NaCl on one side of the membrane and 0.1 *M* KCl on the other side. In the presence of either synthetic or natural valinomycin the observed potential was 150–180 mV (KCl side negative), thus demonstrating the remarkable selectivity for K^+ that is characteristic for valinomycin.

We believe that the solid-phase approach to the synthesis of valinomycin has some advantages over the older

(25) A sample of natural valinomycin was kindly provided by Dr. R. Denkewalter and Dr. F. Holly, Merck Sharp and Dohme Research Laboratories, Rahway, N. J.

Table II. The Effect of Valinomycin on Ionic Permeability of Thin Lipid Membranes

Compound	Concn, *M*	Membrane resistance[a] in KCl (0.1 *M*), ohm cm^2 × 10^{-3}	Biionic potential KCl (0.1 *M*):NaCl (0.1 *M*), mV
None	0	100,000	60–80
Natural valinomycin[b]	10^{-6}	0.3[c]	150
Natural valinomycin	10^{-7}	3.5 (2–5)[d]	178[e]
Synthetic valinomycin	10^{-7}	1.7 (1–3)[f]	175[g]

[a] The numbers in parentheses represent the minimum and maximum resistances observed. [b] Reference 10. [c] Estimated from ref 10, Figure 1. [d] Mean of 16 measurements and 3 different membranes. [e] Mean of 6 measurements and 3 different membranes. [f] Mean of 26 measurements and 4 different membranes. [g] Mean of 6 measurements and 4 different membranes.

methods and that it will be very useful for the synthesis of analogs of this depsipeptide and for the synthesis of depsipeptides in general.

Experimental Section[26]

D-α-Hydroxyisovaleric Acid. A concentrated aqueous solution of 23.2 g (338 mmol) of $NaNO_2$ was added at 0° to a stirred solution of 19.8 g (169 mmol) of D-valine[27] in 338 ml (338 equiv) of 1 *N* H_2SO_4 according to Losse and Bachmann.[28] The crude acid was purified by crystallization from petroleum ether (bp 60–70°): yield 6.4 g (32%), mp 66–67°, $[\alpha]^{20}D$ −16.8° (*c* 1.2, chloroform); lit.[28] (L isomer) mp 63–65°, $[\alpha]^{20}D$ +19.1° (*c* 1.0, chloroform).

Anal. Calcd for $C_5H_{10}O_3$: C, 50.84; H, 8.53. Found: C, 51.00; H, 8.77.

D-α-Hydroxyisovaleric Acid Benzyl Ester. A solution of 23.5 g (198 mmol) of D-α-hydroxyisovaleric acid in 400 ml of benzyl alcohol was saturated with dry hydrochloric acid and allowed to stand at room temperature overnight. After addition of 200 ml of methylene chloride it was washed with water and bicarbonate, dried over Na_2SO_4, and distilled. Fractionation through a 6-in. ring-filled column yielded 35 g, bp 91–93° (0.1 mm); lit.[21] bp 85–90° (0.03 mm). This material contained, by gas chromatography, approximately 15% of an inert compound (presumably cyclo-D-Hyv-D-Hyv).

L-Lactic Acid Benzyl Ester. A 40% (w/v) aqueous solution of L-lactic acid[29] (100 ml) was evaporated under reduced pressure at 30° to 45 ml, diluted with 250 ml of benzyl alcohol, dried over Na_2SO_4 for 2 hr, and filtered. The solution was cooled in a water bath, saturated with dry HCl, allowed to stand at room temperature for 2 hr, diluted with 130 ml of methylene chloride, washed with water and aqueous bicarbonate, dried over Na_2SO_4, and fractionated through a 3-in. ring-filled column. The fraction boiling at 139–141° (14 mm) (lit.[28] bp 138–139° (12 mm)) was pure benzyl L-lactate (by glpc on a SE-30-column); $[\alpha]^{25}D$ −14.7° (*c* 2.8, ethanol); lit.[28] $[\alpha]^{20}D$ −15.0°.

Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.59; H, 6.70.

***t*-Butyloxycarbonyl-D-valine.** The general method of Schnabel[30] (pH-Stat reaction of Boc-azide with amino acids) was used to protect D-valine. After crystallization from petroleum ether (bp 60–70°) the yield was 79%: mp 75.5–76.5°, $[\alpha]^{20}D$ +6.5° (*c* 1.4, glacial acetic acid); lit.[31] for the L isomer: mp 77–79°, $[\alpha]^{25}D$ −5.8° (*c* 1.2, glacial acetic acid).

Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.82; N, 6.45. Found: C, 55.44; H, 8.96; N, 6.44.

***t*-Butyloxycarbonyl-D-valyl-L-lactic Acid.** (a) A solution of 8.45 g (39 mmol) of Boc-D-valine in 25 ml of CH_2Cl_2 was added to 39 ml (39 mmol) of a stirred 1 *M* solution of carbonyldiimidazole in CH_2Cl_2 at 0° over a period of 0.5 hr, followed by 5.4 g (30 mmol) of L-lactic acid benzyl ester. After another hour at 0° the ice bath was removed and the reaction mixture was kept at room temperature for 3 days. At this point analysis by gas chromatography indicated that over 96% of the initially present L-lactic benzyl ester had disappeared. Then 10 ml of water was added to destroy the excess of activated acid and the solvent was removed under reduced pressure. The residue was dissolved in 100 ml of ether and washed with water, citric acid, saturated bicarbonate, and water. After drying over Na_2SO_4 and evaporation of the solvent, the yield was 11.08 g (97%). The pale yellow oil was homogeneous by tlc (silica gel, 70% propanol, R_f 0.82), $[\alpha]^{20}D$ −9.4° (*c* 3.3, benzene). This intermediate (11.0 g, 29 mmol) in 50 ml of methanol was hydrogenated for 18 hr at a pressure of 50 psi in the presence of 2 g of 5% Pd on charcoal. After filtration and evaporation of the solvent the crude acid was dissolved in aqueous bicarbonate, extracted with ether, acidified with citric acid, and extracted into ether. The ether was removed under reduced pressure leaving a colorless clear oil which was dissolved in 10 ml of methanol and stirred into 100 ml of water to yield 6.7 g (80%) of the N-protected didepsipeptide: mp 92.5–93.5°, $[\alpha]^{20}D$ −1.2° (*c* 1, benzene).

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.96; H, 8.01; N, 4.84. Found: C, 54.12; H, 8.09; N, 4.70.

(b) *t*-Butyloxycarbonyl-D-valine (2.72 g, 12.5 mmol) and benzene sulfonyl chloride (1.6 ml, 12.5 mmol) were stirred in 30 ml of dry pyridine at −10° for 10 min according to Shemyakin, *et al.*[20] Then lactic acid benzyl ester (2.25 g, 12.5 mmol) was added. After 20 min at −10° the mixture was allowed to stand at 0° for 1 hr and at room temperature for 4 hr. The product was isolated and purified as previously described.[20] Debenzylation and purification as in part a gave 730 mg (22%), mp 92–93°.

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.96; H, 8.01; N, 4.84. Found: C, 53.90; H, 8.03; N, 4.92.

***t*-Butyloxycarbonyl-L-valyl-D-α-hydroxyisovaleric Acid.** (a) Using essentially the same procedure as described for the synthesis of *t*-butyloxycarbonyl-D-valyl-L-lactic acid (method a), 7.05 g (33 mmol) of Boc-L-Val[27] was activated in 33 ml (33 mmol) of a 1 *M* carbonyldiimidazole solution in CH_2Cl_2 and condensed with 6.25 g (25.5 mmol) of D-α-hydroxyisovaleric acid benzyl ester (85% pure) to give a quantitative yield of a yellow oil which was homogeneous by tlc on silica plates (70% propanol, R_f 0.81), $[\alpha]^{20}D$ +11.1° (*c* 2.8, benzene). This intermediate (10.4 g, 25.5 mmol) was hydrogenated in the presence of 1.5 g of 5% Pd on $BaSO_4$. Crystallization from acetonitrile gave 5.81 g (72%) of colorless needles: mp 140.5–141.0°, $[\alpha]^{20}D$ +1.7° (*c* 1.7, benzene).

Anal. Calcd for $C_{15}H_{27}NO_6$: C, 56.76; H, 8.58; N, 4.41. Found: C, 56.78; H, 8.69; N, 4.38.

(b) Essentially as described before for the preparation of *t*-butyloxycarbonyl-D-valyl-L-lactic acid (method b), 2.62 g (12.1 mmol) of *t*-butyloxycarbonyl-L-valine and 1.61 ml (12.6 mmol) of benzenesulfonyl chloride were combined in 20 ml of pyridine and allowed to react with 2.33 g (11 mmol) of benzyl D-α-hydroxyisovalerate. After 18 hr a second portion of activated acid (2.62 g of *t*-butyloxycarbonyl-L-valine and 1.61 ml of benzene sulfonyl chloride in 10 ml of pyridine) was added at −5°. After 15 min at −5° the mixture was kept at room temperature for 2 hr, then worked-up. The intermediate was debenzylated and purified as in part a to give 420 mg (12%), mp 140.0–140.5°.

Anal. Calcd for $C_{15}H_{27}NO_6$: C, 56.76; H, 8.58; N, 4.41. Found: C, 56.83; H, 8.71; N, 4.30.

***t*-Butyloxycarbonyl-D-valyl-L-lactyl-resin.** *t*-Butyloxycarbonyl-D-valyl-L-lactic acid (2.1 g, 7.75 mmol), triethylamine (1.09 ml, 7.75 mmol), chloromethylated copolystyrene–2% divinylbenzene

(26) Melting points were determined in capillaries and are not corrected. Amino acid analyses (Beckmann Spinco amino acid analyzer 120B) were by Miss Ursula Birkenmaier and elemental analyses by Mr. T. Bella of Rockefeller University. Optical rotations were determined in the laboratories of Hoffmann La Roche, Inc., Nutley, N. J., through the courtesy of Dr. J. A. W. Gutzwiller and Dr. A. M. Felix. Thin-layer chromatography was essentially performed as depicted in G. Pataki, "Dünnschichtchromatographie in der Aminosäure und Peptidchemie," Walter de Gruyter & Co., Berlin, 1966.

(27) Grade 1, Cyclo Chem. Corp., Los Angeles, Calif.

(28) G. Losse and G. Bachmann, *Chem. Ber.*, **97**, 2671 (1964).

(29) By Schwarz BioResearch, Inc., Orangeburg, N. Y.

(30) E. Schnabel, *Ann.*, **702**, 188 (1967).

(31) G. W. Anderson and A. C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).

beads (6.0 g, 11.6 mequiv, 200–400 mesh),[29] and ethyl acetate (45 ml) were refluxed for 51 hr. The resin was then filtered and washed with ethyl acetate, ethanol, water, ethanol, glacial acetic acid, ethanol, and methylene chloride. The dried (40°, 20 hr, 0.05 mm) resin weighed 7.50 g. Hydrolysis (refluxing 24 hr in dioxane–concentrated HCl, 1:1, v/v) of an aliquot of the resin gave 0.83 mmol of valine/g which corresponds to a yield of 80% for the esterification step. A second preparation contained 0.94 mmol/g.

t-Butyloxycarbonyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactyl-L-valyl-D-α hydroxyisovaleryl-D-valyl-L-lactyl-resin. *t*-Butyloxycarbonyl-D-valyl-L-lactyl-resin (1.17 g; containing 1.1 mmol of protected didepsipeptide) was placed in the reaction vessel of the instrument for automated synthesis of peptides described by Merrifield, Stewart, and Jernberg.[24] The instrument was programmed and and equipped to perform automatically the following coupling cycle: three 1.5-min washes with dioxane; deprotection with 4 *N* HCl in dioxane (30 min); three 1.5-min washes each with dioxane, ethanol, and chloroform; neutralization with triethylamine (10% in chloroform), 10 min; three 1.5-min washes each with chloroform and methylene chloride; soaking of the resin for 10 min with a solution of 2.2 mmol of the proper coupling unit in methylene chloride (*t*-butyloxycarbonyl-L-valyl-D-α-hydroxyisovaleric acid, 0.70 g, or *t*-butyloxycarbonyl-D-valyl-L-lactic acid, 0.63 g); addition of 0.452 g (2.2 mmol) of N,N′-dicyclohexylcarbodiimide[32] in methylene chloride and shaking for 4 hr; three 1.5-min washes with methylene chloride and two with ethanol. This cycle was repeated five times as indicated in Figure 3, with minor modifications in the first coupling step where 4.4 mmol of coupling unit and DCC were used and the ethanol wash after deprotection was omitted.

Analysis of the Depsipeptide Intermediates. The synthesis was monitored in three ways. During the first coupling step the considerable increase of absorbtion in the carbonyl region (1675–1780 cm^{-1}) of the ir spectrum was a qualitative indication of satisfactory coupling. Samples of the depsipeptide-resin were hydrolyzed for 4–5 days in dioxane–concentrated HCl (1:1, v/v) in a sealed vessel at 110° and the valine liberated was quantitatively determined. The increase of the valine content of the resin was found to be between 80 and 110% of the calculated values for each coupling step. The intermediate depsipeptides were cleaved from small samples of the resin by HBr in trifluoroacetic acid (as described below), and were checked for purity by tlc (Table I).

L-Valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactic Acid Acetate. The resin was suspended in anhydrous trifluoroacetic acid (TFA) and a steady stream of HBr was bubbled through for 90 min at room temperature with exclusion of moisture. The suspension was filtered and the filtrate, after combination with three additional TFA washes of the resin, was evaporated at 30° under reduced pressure. The product was dissolved in 3 ml of warm glacial acetic acid and stirred into 40 ml of water. The crude open-chain valinomycin acetate that precipitated was separated from the aqueous layer and dried under reduced pressure over KOH; amorphous yellowish mass, 830 mg, 64%. It was homogeneous by tlc (see Table I for R_f value), and free of halogen but still contained some trifluoroacetate (by fluorine analysis). A sample of the crude product was dissolved in methanol, neutralized with triethylamine, and precipitated with water. It was dissolved in acetic acid, reprecipitated in water and finally lyophylized from acetic acid to give the acetate monohydrate: mp 110–114°, $[\alpha]^{20}D$ +14.1° (*c* 0.8, acetic acid).

Anal. Calcd for $C_{56}H_{98}N_6O_{22}$: C, 55.75; H, 8.19; N, 6.97. Found: C, 55.79; H, 8.07; N, 6.96.

Valinomycin. Following directions of Shemyakin, *et al.*,[20] for the cyclization of tetradepsipeptides, 280 mg (0.212 mmol, by valine analysis) of open-chain valinomycin acetate was dissolved in 1 ml of thionyl chloride and kept at room temperature for 30 min. The solution was evaporated at 30° under reduced pressure and re-evaporated several times with addition of absolute benzene.[33] The resulting acid chloride was dissolved in 25 ml of absolute benzene and dropped simultaneously with 0.075 ml (0.530 mmol) of triethylamine in 25 ml of absolute benzene into 125 ml of stirred absolute benzene over a period of 3 hr at room temperature.[34] After 17 hr at room temperature 0.1 ml of triethylamine was added, and after another 90 min the solvent was removed under reduced pressure. The solid residue was dissolved in ether, separated by filtration from a minor precipitate, and evaporated (264 mg of solid). It was again dissolved in ether, washed with 1 *N* HCl, aqueous bicarbonate, and water, dried over Na_2SO_4, and evaporated to 2 ml. Upon addition of 10 ml of petroleum ether (bp 30–60°) a small precipitate formed and was filtered; the filtrate was evaporated and yielded 194 mg of crude crystalline valinomycin (82%), mp 178–182°. It was recrystallized from *n*-butyl ether to give 100 mg (mp 185–186°). Another 20 mg (mp 183–185°) was isolated from the mother liquor after evaporation and crystallization from nitromethane. Both fractions were pure by elemental analysis. They gave one spot in five different systems in tlc (for R_f values see Table I), which was ninhydrin negative and pale yellow in the iodine–tolidine reaction. Total yield (120 mg) was 51% for the cyclization step and 33% over-all based on the starting material, Boc-D-Val-L-Lac-resin. The fraction with a melting point of 185–186° had a specific optical rotation of $[\alpha]^{20}D$ +31.8° (*c* 1, benzene), lit. +31.0° [2] and 32.8° [5] and a valine content of 5.89 μmol/mg (calcd 5.90). The ir spectra of the synthetic and natural product[25] were identical with that published by Brockmann.[2] There was no mixture melting point depression with the natural antibiotic (mp 186.0–186.5°).[25] Two additional crystallizations from nitromethane raised the melting point to 186.5–187.0°, lit. 190° [2] and 187°.[5]

Anal. Calcd for $C_{54}H_{90}N_6O_{18}$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.34; H, 8.23; N, 7.40.

Acknowledgment. We wish to thank Dr. M. Tieffenberg of Duke University for the measurements on membranes.

(32) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955).

(33) Commercial thiophene-free benzene was distilled under anhydrous conditions after refluxing over sodium and addition of sodium hydride.

(34) According to more recent experiments, cyclization of this depsipeptide does not require high dilution. Furthermore, indications are that ring closure of the dodecadepsipeptide chloride hydrochloride occurs spontaneously without addition of base.