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Polyoxin Analogs. III.[1] Synthesis and Biological Activity of Aminoacyl Derivatives of Polyoxins C and L

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A number of aminoacyl derivatives of nucleoside antibiotics, polyoxins C and L were prepared. Aminoacylation was performed in aqueous dimethylformamide with an equimolar N-carbobenzoxyamino acid *p*-nitrophenyl ester in the presence of an equimolar triethylamine or N-methylmorpholine. Catalytic hydrogenolysis of the condensation product followed by chromatographic purification afforded the aimed compounds in moderate yields. Antifungal activities were tested with several lines of phytopathogenic fungi, which led to some conclusion upon structure-activity relationship regarding the N terminal amino acid: (1) the α-L-amino group is essential for the activity, (2) the alkyl chain length and especially nature of the ω-substituents affect considerably the activity. Many of the aminoacyl derivatives of polyoxin L retained moderate activity. None of the compounds prepared showed broader antimicrobial spectrum than the natural polyoxins.

Keywords——nucleoside peptide antibiotic; polyoxin C, aminoacyl derivatives of; polyoxin L, aminoacyl derivatives of; aminoacylation; DL-α-amino-δ-carbamoyloxyvaleric acid; structure-activity relationship; antifungal activity; cell-wall chitin biosynthesis

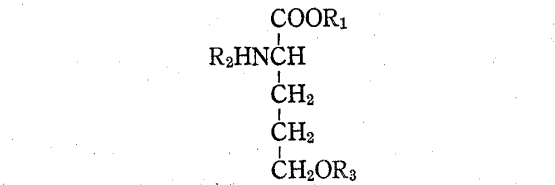
The polyoxins represnt a group of pyrimidine nucleoside peptide antibiotics which are elaborated by *Streptomyces cacaoi* var. *asoensis*.[3] The structural similarity with UDP-N-acetylglucosamine was proposed to be the basis of their selective inhibitory properties towards chitin synthetase of fungi and yeasts.[3] Indeed, the kinetics of inhibition for chitin synthetases from various sources were all shown to be of competitive type.[4] We have attempted the synthesis of a number of polyoxin analogs for the purpose of (1) elucidation of structure-activity relationship; (2) overcoming the transport barrier of some yeasts and fungi which resist to the polyoxins; and (3) modification of the polyoxin molecule so as to inhibit the bacterial cell wall biosynthesis. The last possibility is based on the fact that UDP-N-acetylglucosamine is also an intermediate in bacterial cell wall biosynthesis. Total synthesis of polyoxin J was achieved.[5] However, no synthetic analogs were prepared.

On this line of the study, we have already reported[6] the synthesis of the aminoacyl derivatives of 5′-amino-5′-deoxyuridine with the conclusion that the carboxyl group on carbon-5′ of the polyoxins is absolutely required for the activity. A preliminary account has also been published concerning the preparation and biological activity of some aminoacyl derivatives of polyoxin C, in which the structure-activity relationship has been discussed.[1] Further, Hori, *et al.*[7] determined *in vitro* activity of some of these compounds using chitin synthetase

1) Part II: K. Isono, S. Suzuki, and T. Azuma, *Agric. Biol. Chem.* (Tokyo), **35**, 1986 (1971).
2) Location: *Hirosawa, Wako-shi, Saitama, 351, Japan.*
3) K. Isono, K. Asahi, and S. Suzuki, *J. Am. Chem. Soc.*, **91**, 7490 (1969).
4) *a*) A. Endo and T. Misato, *Biochem. Biophys. Res. Commn.*, **37**, 718 (1969); *b*) F.A. Keller and E. Cabib, *J. Biol. Chem.*, **246**, 160 (1971); *c*) M. Hori, K. Kakiki, S. Suzuki, and T. Misato, *Agric. Biol. Chem.* (Tokyo), **35**, 1280 (1971); *d*) M. Hori, J. Eguchi, K. Kakiki, and T. Misato, *J. Antibiotics*, **27**, 260 (1974); *e*) Y.N. Jan, *J. Biol. Chem.*, **249**, 1973 (1974); *f*) S. Bartnicki-Garcia and E. Lippman, *J. Gen. Microbiol.*, **71**, 301 (1972).
5) H. Kuzuhara, H. Ohrui, and S. Emoto, *Tetrahedron Lett.*, **1973**, 5055.
6) K. Isono, T. Azuma, and S. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **19**, 505 (1971).
7) M. Hori, K. Kakiki, and T. Misato, *Agric. Biol. Chem.* (Tokyo), **38**, 691 (1974).

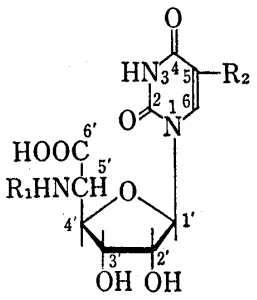
from *Piricularia oryzae*. The present paper deals with in full detail the preparation and biological activities of these aminoacyl derivatives of polyoxin C (**6**) including six additional derivatives. Furthermore, aminoacyl derivatives of polyoxin L (**23**) were also prepared for the evaluation of their biological activities.

Synthesis of aminoacyl derivatives was performed by the reaction of *p*-nitrophenyl esters of N-carbobenzoxyamino acids with polyoxin C in the presence of an equimolar of triethylamine or N-methylmorpholine in an aqueous dimethylformamide (DMF). DL-α-Amino-δ-carbamoyloxyvaleric acid (**4**) which represents 3,4-dideoxy analog of natural carbamoylpolyoxamic acid,[3] was synthesized from N-tosyl-α-L-amino-δ-hydroxyvaleric acid[8] (**1**) by benzyl esterification, carbamoylation, followed by Birch reduction.[9] It is also of



	R_1	R_2	R_3
1:	H	*p*-toluenesulfonyl-	H
2:	benzyl-	*p*-toluenesulfonyl-	H
3:	benzyl-	*p*-toluenesulfonyl-	$CONH_2$
4:	H	H	$CONH_2$
5:	*p*-nitrophenyl-	carbobenzoxy-	$CONH_2$

Chart 1



compound	R_1	R_2
6 :	–H	$-CH_2OH$
7a:	$-COCH_2NH_2$	$-CH_3$
7b:	$-COCH_2NH_2$	$-CH_2OH$
8 :	$-COCH(NH_2)CH_3$ (L)	$-CH_3$
9 :	$-COCH(NH_2)CH_3$ (D)	$-CH_3$
10 :	$-COCH_2CH_2NH_2$	$-CH_3$
11 :	$-COCH(NH_2)CH_2CH_2CH_3$ (L)	$-CH_3$
12 :	$-COCH(NH_2)CH_2CH_2CH_3$ (D)	$-CH_3$
13 :	$-COCH(NH_2)CH_2CH_2CH_2NH_2\cdot 2HCl$ (L)	$-CH_2OH$
14 :	$-COCH(NH_2)CH_2CH_2CH_2CH_2NH_2\cdot 2HCl$ (L)	$-CH_2OH$
15 :	$-COCH(NH_2)CH_2CH_2COOH$ (L)	$-CH_3$
16 :	$-COCH(NH_2)CH_2CH_2CONH_2$ (L)	$-CH_3$
17a:	$-COCH(NH_2)CH_2CH_2CH_2NHCOCH_3$ (L)	$-CH_3$
17b:	$-COCH(NH_2)CH_2CH_2CH_2NHCOCH_3$ (L)	$-CH_2OH$
18 :	$-COCH(NH_2)CH_2CH_2CH_2NHCONH_2$ (L)	$-CH_3$
19a:	$-COCH(NH_2)CH_2CH_2CH_2OCONH_2$ (DL)	$-CH_3$
19b:	$-COCH(NH_2)CH_2CH_2CH_2OCONH_2$ (DL)	$-CH_2OH$
20 :	$-COCH(NH_2)CH_2Ph$ (L)	$-CH_2OH$
21 :	$-COCH(NH_2)CH_2CH_2CH_2NHCOC_{10}H_{15}$ (L)	$-CH_2OH$
22 :	$-COCH(NH_2)CH_2CH_2CH_2NHCOC_{11}H_{23}$ (L)	$-CH_2OH$
polyoxin J:	$-COCH(NH_2)CH(OH)CH(OH)CH_2OCONH_2$ (L)	$-CH_3$

Ph=phenyl

Chart 2

8) M. Goodman and A.M. Felix, *Biochemistry*, 3, 1529 (1964).

9) Racemization took place, when N-tosyl-L-α-amino-δ-hydroxyvaleric acid was esterified with benzyl alcohol in the presence of *p*-toluenesulfonic acid. To avoid this, the corresponding N-carbobenzoxy derivatives were used for benzyl esterification. However, the subsequent carbamoylation with phosgene and ammonia again resulted in racemization. Thus the α-amino-δ-carbamoyloxyvaleroyl derivatives of polyoxin C (**19a** and **19b**) are considered to be diastereomeric mixtures, which were suggested by the broad signals in nuclear magnetic resonance (NMR) spectra. Mechanism of the latter racemization is not known.

biosynthetic interest since it has recently been shown to be an intermediate amino acid for the biosynthesis of carbamoylpolyoxamic acid.[10] Condensation products, after deblocked by hydrogenolysis[11] over palladium in aqueous methanol, were purified by cellulose chromatography. Compounds prepared (**7**—**22**) are listed in Chart 2. Assignable protons of ^{1}H NMR spectra were listed in Table I, which supports the presence of both the nucleoside moiety and the side chain amino acids.

Table I. ^{1}H NMR Spectral Data of Aminoacyl Derivatives of Polyoxin C (**6**) (δ in D_2O)

Compound	Pyrimidine		Sugar		Aminoacyl group[d]
	H-6[a]	CH_3 on C-5[b]	H-1′[c]	$J_{1',2'}$	
7a	7.43	1.95	5.95	4.4	3.95 (s, 2H, 2″-C<u>H</u>$_2$)
7b	7.60		5.85	4.0	3.96 (s, 2H, 2″-C<u>H</u>$_2$)
8	7.57	1.94	5.88	5.8	1.61 (d, 3H, 3″-C<u>H</u>$_3$, J=7.0)
9	7.52	1.92	5.88	5.1	1.56 (d, 3H, 3″-C<u>H</u>$_3$, J=7.2)
10	7.64	2.04	6.00	5.4	2.91 (br t, 2H, 2″-C<u>H</u>$_2$), 3.44 (br t, 2H, 3″-C<u>H</u>$_2$)
11	7.60	1.96	5.90	5.0	1.00 (t, 3H, 5″-C<u>H</u>$_3$, J=6.6), 1.3—1.7 (m, 2H, 4″-C<u>H</u>$_2$), 1.8—2.1 (m, 2H, 3″-C<u>H</u>$_2$)
12	7.62	1.96	5.92	5.1	1.3—1.7 (m, 2H, 4″-C<u>H</u>$_2$), 1.7—2.2 (m, 2H, 3″-C<u>H</u>$_2$)
13	7.81		5.94	4.8	1.6—2.3 (m, 4H, 3″,4″-C<u>H</u>$_2$), 3.13 (br t, 5″-C<u>H</u>$_2$)
14	7.54		5.88	5.3	1.3—2.0 (m, 6H, 3″,4″,5″-C<u>H</u>$_2$), 3.02 (br t, 2H, 6″-C<u>H</u>$_2$)
15	7.54	1.92	5.86	5.2	2.22[e] (m, 2H, 3″-C<u>H</u>$_2$), 2.58[e] (m, 2H, 4″-C<u>H</u>$_2$)
16	7.60	1.94	5.89	5.4	2.16 (br t, 2H, 3″-C<u>H</u>$_2$), 2.53 (br t, 2H, 4″-C<u>H</u>$_2$)
17a	7.60	1.91	5.86	6.2	1.98 (s, 3H, C<u>H</u>$_3$CO), 1.5—2.1 (m, 4H, 3″,4″-C<u>H</u>$_2$), 3.11 (br t, 2H, 5″-C<u>H</u>$_2$)
17b	7.77		5.89	5.1	1.5—2.1 (m, 4H, 3″,4″-C<u>H</u>$_2$), 2.00 (s, 3H, C<u>H</u>$_3$CO), 3.24 (br t, 2H, 5″-C<u>H</u>$_2$)
18	7.54	1.92	5.85	5.4	1.4—2.1 (m, 4H, 3″,4″-C<u>H</u>$_2$), 3.15 (br t., 2H, 5″-C<u>H</u>$_2$)
19a	7.60	1.95	5.87	5.9	1.6—2.3 (m, 4H, 3″,4″-C<u>H</u>$_2$)
19b	7.79		5.93	5.0	1.7—2.2 (m, 4H, 3″,4″-C<u>H</u>$_2$)
20	7.73		5.87	4.4	7.20[e] (m, 5H, aromatic H)
21	7.72		5.90	6.0	1.4—2.1 (m, 19H, 3″,4″-C<u>H</u>$_2$ and C<u>H</u>, C<u>H</u>$_2$ of adamantoyl), 3.06[e] (m, 2H, 5″-C<u>H</u>$_2$)
22[f]	7.72		5.80	6.0	0.98 (br t, 3H, C<u>H</u>$_3$ of lauroyl), 1.1—1.8 (m, 22H, 3″,4″-C<u>H</u>$_2$ and C<u>H</u>$_2$ of lauroyl), 2.07 (m, 2H, -C<u>H</u>$_2$CONH of lauroyl), 3.06 (m, 2H, 5″-C<u>H</u>$_2$)

a) Broad singlet, 1H.
b) Broad singlet, 3H.
b) Doublet, 1H.
d) Numbering of N terminal amino acids was expressed by double prime.
e) Apparent center of broad multiplet.
f) In DMSO-d_6–D_2O.

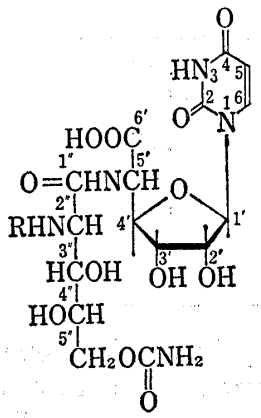
10) K. Isono and S. Funayama, 10th International Symposium on the Chemistry of Natural Products, Abstract Papers D3 (1976).
11) In most cases except basic amino acid derivatives (**13**, **14**), hydrogenolysis of the hydroxymethyl group on carbon-5 of uracil to give thymine derivatives was observed.

Table II. Antifungal Activity[a] of Aminoacyl Derivatives of Polyoxin C

Compound	Minimal inhibitory concentration (mcg/ml)		
	Alternaria kikuchiana	*Piricularia oryzae*	*Cochliobolus miyabeanus*
6	>200	>200	>200
7a	>200[b]	>200	>200[b]
8	>200[b]	50	>200[b]
9	>200	>200	>200
10	>200	>200	>200
11	200	25	>200
12	>200	>200	>200
13	25	200	>200
14	>200	>200	>200
15	>200	>200	>200
16	100	50	>200[b]
17a	100	12.5	100
18	6.25	12.5	100
19a	25	12.5	50
20	>200	200	>200
21	>200	>200	>200
22	>200	>200	100
Polyoxin J	25	25	12.5

a) Conventional agar dilution technique was employed.
b) Partial inhibition was observed at this concentration.

Inhibitory activity of aminoacyl derivatives of polyoxin C towards several species of phytopathogenic fungi is shown in Table II, from which the following conclusion may be drawn. (1) Free N-terminal amino group on the α-carbon is absolutely required for the activity. None of the acyl derivatives and β-aminoacyl derivative were active. (2) Configuration at the α-carbon must be L. D-Alanyl (**9**), D-α-aminovaleroyl (**12**) derivatives were inactive in contrast to the corresponding L-derivatives. (3) Alkyl chain length of amino acids affects the activity. Activity decreases as the chain length decreases. (4) ω-Substituents are also important for the activity. Polar groups such as acetamide (**17a, 17b**), ureido (**18**), carbamoyl (**16**), and carbamoyloxy (**19a, 19b**) groups increase the activity. In contrast, the ionic groups such as amino (**13, 14**) or carboxyl (**15**) significantly depress the activity. Introduction of aromatic group (**20**) exerted unfavorable effect for the activity, which is in contrast to *in vitro* data.[7]



compound	R
23:	–H
24:	$-COCH_2NH_2$
25:	$-COCH(NH_2)CH_3$ (L)
26:	$-COCH(NH_2)CH_3$ (D)
27:	$-COCH(NH_2)CH_2CH_2CH(CH_3)_2$ (L)
28:	$-COCH(NH_2)CH_2Ph$ (L)
29:	$-COCH(NH_2)CH_2CH_2CH_2NHCOC_{10}H_{15}$ (L)
30:	$-COCH(NH_2)CH_2CH_2CH_2NHCOC_{11}H_{23}$ (L)

Ph=phenyl

Chart 3

Some aminoacyl derivatives of polyoxin L (**23**) were also prepared. We expected a favorable change in permeability by lengthening the peptide chain by amino acids having a hydrophobic group. Synthetic procedure used is essentially the same to those used for aminoacyl

Table III. ^{1}H NMR Spectral Data of Aminoacyl Derivatives of Polyoxin L (23) (δ in D_2O)

Compund	Pyrimidine H-5[a)]	Pyrimidine H-6[a)]	Pyrimidine $J_{5,6}$	Sugar H-1′	Sugar $J_{1',2'}$	Aminoacyl group
24	5.95	7.68	8.3	5.92	4.4	4.01 (s, 2H, 2‴-C$\underline{H}_2$)
25	5.96	7.66	8.0	5.90	3.8	1.62 (d, 3H, 3‴-C$\underline{H}_3$, J=7.4)
26	5.95	7.66	7.8	5.90	4.0	1.61 (d, 3H, 3‴-C$\underline{H}_3$, J=7.6)
27	6.02	7.74	8.2	5.97	5.0	1.06[c)] (m, 6H, two C$\underline{H}_3$ on C-4‴), 2.82[c)] (m, 2H, 3‴-C$\underline{H}$)
28	5.58	7.62	8.2	5.94	6.0	3.09, 3.33 (ABq, $C_6H_5C\underline{H}_2$, J=14.0)
29	5.97	7.63	8.4	5.88	4.4	1.5—2.2 (m, 19H, 3‴, 4‴-C$\underline{H}_2$ and C$\underline{H}$, C$\underline{H}_2$ of adamantoyl)
30	5.85	7.65	8.2	5.90	4.4	0.85 (br t, 3H, C$\underline{H}_3$ of lauroyl), 1.1—1.9 (m, 22H, 3‴, 4‴-C$\underline{H}_2$ and C$\underline{H}_2$ of lauroyl), 2.11[c)] (m, 2H, -C$\underline{H}_2$CONH of lauroyl) 3.11[c)] (m, 2H, 5‴-C$\underline{H}_2$)

a) Doublet, 1H.
b) Numbering of N terminal amino acids was expressed by triple prime.
c) Apparent center of broad multiplet.

Table IV. Antifungal Activity of Aminoacyl Derivatives of Polyoxin L

Compound	Minimal inhibitory concentration (mcg/ml) *Alternaria kikuchiana*	*Piricularia oryzae*	*Cochlibolus miyabeanus*	*Sclerotinia cinerea*	*Pellicularia sasakii*
23	12.5	6.25	3.12	12.5	25
24	50	50	50	50	>100
25	12.5	12.5	12.5	25	
26	50	12.5	12.5	100	50
27	25	6.25	12.5	50	
28	50	50	25	100	25
29	>100	100	>100	>100	100
30	100	25	50	50	100

derivatives of polyoxin C. Compounds prepared are listed in Chart 3 (**24**—**29**). ^{1}H NMR spectra data were listed in Table III, which support the structures. In general, they exerted moderate antifungal activity (Table IV). D-Alanyl derivative (**26**) was almost as equally active as L-alanyl derivative (**25**). This is in contrast to the corresponding derivatives of polyoxin C (**8** and **9**). It may be that either the hydrolysis of N-terminal amino acid occurs in cells, or there is another negative center at the binding site of chitin synthetase in a position to bind with a new amino group. L-Leucyl derivative (**27**) may also be interesting from the stand point of biosynthesis, since an aminoacyl nucleoside antibiotic, bleasticidin S was reported to be formed from a precursor, N-leucylblasticidin S.[12)]

In order to increases cell-membrane permeability, lypophilic lauroyl and adamantoyl[13)] groups were introduced into the δ-amino group of ornithine (**21**, **22**, **29**, **30**). However, this attempt resulted in considerable decrease in activity. It may be that the large alkyl groups prevent sterically access of the molecule into the binding site of the enzyme. All the compounds prepared were also examined their effects towards several species of bacteria. None of them showed significant antibacterial activity.

12) H. Seto, N. Otake, and H. Yonehara, *Agric. Biol. Chem.* (Tokyo), **32**, 1299 (1968).
13) Biological significance of this group was discussed. See K. Gerzon and D. Kau, *J. Med. Chem.*, **10**, 189 (1967) and references cited therein.

Experimental

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were run on a Perkin-Elmer 521 grating infrared spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. NMR spectra were run on a Varian HA-100 NMR spectrophotometer. Chemical shifts were measured to an internal or external standard, tetramethylsilane (TMS), and are recorded as δ values.[14] Coupling constants were expressed in Hz. Thin-layer chromatography was developed on either Silica Gel G (E. Merck AG) or Avicel SF microcrystalline cellulose. 2′,3′-Diols were detected with a periodate-benzidine spray reagent[15] and amino groups were detected with a ninhydrin test. Mallinckrodt's Silicic Acid AR and Avicel microcrystalline cellulose were used for column chromatography. All the aminoacyl derivatives for elemental analysis were dried over P_2O_5 at 100° for 2 hr.[16] Solvent system used for cellulose column chromatography is; butanol–acetic acid–water (4: 1: 1—4: 1: 1.5). Polyoxin C (**6**) was prepared by alkaline hydrolysis of the polyoxin complex as described before.[3] Polyoxin L (**22**) was prepared by bisulfite catalyzed decarboxylation[17] of polyoxin D.

***N*-Tosyl-DL-α-amino-δ-hydroxyvaleric Acid Benzyl Ester (2)**——A suspensoin of 3.77 g of *N*-tosyl-L-α-amino-δ-hydroxyvaleric acid[8] (**1**) in 130 ml of anhydrous benzene was refluxed azeotropically for 5 hr in the presence of 2.1 g of benzyl alcohol and 0.2 g of *p*-toluenesulfonic acid. The reaction solution was washed twice with 5% $KHCO_3$ (each 10 ml) and finally with water. It was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to dryness. The resulting colorless syrup was subjected to silica gel chromatography (benzene–acetone, 5: 1). A colorless syrup (962 mg) of **2** was obtained. IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O). NMR (acetone-d_6) δ: 1.65 (4H, m, 3,4-C$\underline{H}_2$), 2.39 (3H, s, C$\underline{H}_3$), 3.48 (2H, diffused t, 5-C$\underline{H}_2$), 3.95 (1H, m, 2-C$\underline{H}$), 4.88 (2H, s, benzyl-C$\underline{H}_2$), 7.2—7.8 (9H, aromatic $\underline{H}$). MS *m/e*: 269 (M–C_7H_8O). $[\alpha]_D^{20}$ 0° (*c*=1.6, EtOH).

***N*-Tosyl-DL-α-amino-δ-carbamoyloxyvaleric Acid Benzyl Ester (3)**——To an ice-cooled solution of 590 mg of **2** in 6 ml of anhydrous toluene, was added dropwise 20 ml of 8.5% phosgene solution in toluene with stirring for 30 min. After standing additional 2 hr at room temperature, the reaction solution was concentrated *in vacuo* to a small volume. The residue was cooled in an ice bath and 7 ml of ice-cooled conc. NH_4OH was added with stirring. After stirring for few minutes, the reaction mixture was concentrated to dryness. The resulting colorless syrup was dissolved in ethyl acetate and the solution was washed with water. After dehydration with Na_2SO_4, it was concentrated to dryness. The residual syrup was crystallized from ether affording 470 mg of **3** as colorless crystals, mp 117—121°. NMR (acetone-d_6) δ: 1.70 (4H, m, 3,4-C$\underline{H}_2$), 2.40 (3H, s, C$\underline{H}_3$), 3.91 (2H, diffused t, 5-C$\underline{H}_2$), 4.90 (2H, s, benzyl-C$\underline{H}_2$), 5.80 (2H, broad, OCONH$_2$), 7.2—7.8 (9H, aromatic H).

DL-α-Amino-δ-carbamoyloxyvaleric Acid (4)——To a solution of 432 mg of **3** in 30 ml of anhydrous liquid ammonia, sodium was added with stirring until blue color lasted for more than 3 min, which was then discharged by the addition of ammonium acetate. After ammonia was evaporated off, the residue was dissolved in 50 ml of water and passed through a column of Dowex 50 (H^+). The column was washed with water, then eluted with 3% NH_4OH. Ninhydrin positive fractions were combined and concentrated to a small volume and treated with ethanol, affording 65 mg of **4** as colorless crystals, mp 248—250° (dec.). $[\alpha]_D^{20}$ 0° (*c*=1.00, H_2O). *Anal.* Calcd. for $C_6H_{12}N_2O_4$: C, 40.90; H, 6.87; N, 15.90. Found: C, 40.43; H, 6.70; N, 15.42.

***N*-Carbobenzoxy-DL-α-amino-δ-carbamoyloxyvaleric Acid *p*-Nitrophenyl Ester (5)**——Conventional Schotten-Baumann procedure was employed for the preparation of the carbobenzoxyamino acid, mp 123°. *p*-Nitrophenyl ester was prepared from equimolar *p*-nitrophenol and dicyclohexylcarbodiimide (DCC) in DMF. The product **5** was crystallized from ethanol as colorless needles, mp 156°. *Anal.* Calcd. for $C_{20}H_{21}N_3O_8$: C, 55.68; H, 4.91; N, 9.74. Found: C, 55.56; H, 4.82; N, 9.71.

5′-N-Glycylthyminepolyoxin C (7a) and 5′-N-Glycylpolyoxin C (7b)——To an ice-cooled solution of 503 mg of **6** and 0.195 ml of triethylamine in a mixture of water (20 ml) and DMF (20 ml), was added a solution of 495 mg of carbobenzoxyglycine *p*-nitrophenyl ester in 10 ml of DMF. After standing overnight at 37°, the reaction mixture was passed through a column of Dowex 50 (H^+) (3 ml). Effluent was concentrated *in vacuo* to a small volume. The product was precipitated by the addition of ether; amorphous powder, 380 mg. It was dissolved in 10 ml of water and the solution was hydrogenated over 60 mg of palladium black at atmospheric pressure for 3 hr. The products were purified by cellulose chromatography. From the first fraction, 53 mg of **7a** was obtained after recrystallization from aqueous ethanol; mp 220—225° (dec.), $[\alpha]_D^{20}$ +15.1° (*c*=1.116, 0.1 N HCl). *Anal.* Calcd. for $C_{13}H_{18}N_4O_8 \cdot H_2O$: C, 41.49; H, 5.36; N, 14.89. Found: C, 41.63; H, 5.13; N, 14.77. From the second fraction, 95 mg of crystals of **7b** was obtained; mp 220—260°

14) Multiplicity of signals were expressed by the following abbreviation: s, singlet; d, doublet; t, triplet; m, multiplet; br; broad.

15) R.U. Lemieux and H.F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

16) In many cases, solvation of water and ethanol was too strong to be removed completely under this condition. Solvation of ethanol was confirmed by ^1H NMR spectrum (δ 2.20, t, –C$\underline{H}_3$ and 3.67, q, –C$\underline{H}_2$).

17) K. Shibuya, M. Tanaka, T. Nanbata, K. Isono, and S. Suzuki, *Agric. Biol. Chem.* (Tokyo), **36**, 1229 (1972).

(dec.), $[\alpha]_D^{20}$ +20.8° (c=1.008, 0.1 N HCl). *Anal.* Calcd. for $C_{13}H_{18}N_4O_9 \cdot 1/4\ H_2O$: C, 41.22; H, 4.92; N, 14.79. Found: C, 41.19; H, 4.40; N, 14.72.

5′-N-L-Alanylthyminepolyoxin C (8)——Polyoxin C (**6**) (503 mg), triethylamine (0.195 ml), and carbobenzoxy-L-alanine *p*-nitrophenyl ester (518 mg) were reacted as in the preceding section. Product (300 mg) was hydrogenated over palladium (50 mg). After cellulose chromatography, 150 mg of **8** was obtained as crystalline powder, mp 200—205° (dec.), $[\alpha]_D^{20}$ +32.8° (c=1.014, H_2O). *Anal.* Calcd. for $C_{14}H_{20}N_4O_8 \cdot 1/4H_2O$: C, 44.44; H, 5.48; N, 14.81. Found: C, 44.69; H, 5.53; N, 14.60.

5′-N-D-Alanylthyminepolyoxin C (9)——Reaction was performed in the similar way as in the preparation of **8**. Product **9** was obtained as colorless powder; 140 mg, mp 200—205° (dec.), $[\alpha]_D^{20}$ +14.0° (c=1.000, H_2O). *Anal.* Calcd. for $C_{14}H_{20}N_4O_8 \cdot 1/4H_2O$: C, 44.44; H, 5.48; N, 14.81. Found: C, 44.82; H, 5.50; N, 14.65.

5′-N-β-Alanylthyminepolyoxin C (10)——To a solution of 335 mg of **6** and 111 mg of N-methylmorpholine in a mixture of water (20 ml) and DMF (13 ml), was added a solution of 345 mg of N-carbobenzoxy-β-alanine *p*-nitrophenyl ester in 14 ml of DMF. The solution was allowed to stand overnight at 37°. The reaction mixture, after passing through Dowex 50 (H^+) column, was concentrated to a small volume and treated with ethanol–ether, affording 286 mg of amorphous powder. It was dissolved in 160 ml of 50% methanol and hydrogenated over 28 mg of palladium black for 2 hr. After work-up as described, 155 mg of **10** was obtained as crystals from aqueous ethanol; mp 260—265° (dec.), $[\alpha]_D^{20}$ +20.3° (c=1.00, H_2O). *Anal.* Calcd. for $C_{14}H_{22}N_4O_9 \cdot H_2O$: C, 43.07; H, 5.68; N, 14.35. Found: C, 42.85; H, 5.25, N, 14.29.

5′-N-L-α-Aminovaleroylthyminepolyoxin C (11)——To an ice-cooled solution of 670 mg of **6** in a mixture of 30 ml of 33% aqueous DMF and 0.3 ml of triethylamine was added a solution of 745 mg of N-carbobenzoxy-L-α-aminovaleric acid *p*-nitrophenyl ester in 20 ml of DMF and the solution was stirred for 1 hr in an ice-bath and additional 2 days at room temperature. After work-up, 742 mg of colorless plates was obtained; mp 133—137°. The crystals (200 mg) were dissolved in 17% methanol and hydrogenated over 20 mg of palladium black for 2 hr. After work-up, 120 mg of **11** was obtained as white powder; mp 192—195° (dec.). $[\alpha]_D^{20}$ +32.9° (c=1.260, H_2O). *Anal.* Calcd. for $C_{16}H_{24}N_4O_8 \cdot 1/2H_2O$: C, 46.96; H, 6.11; N, 13.69. Found: C, 46.80; H, 6.02; N, 13.50.

5′-N-D-α-Aminovaleroylthyminepolyoxin C (12)——To a solution of 335 mg of **6** in a mixture of 20 ml of water, 13 ml of DMF, and 111 mg of N-methylmorpholine was added a solution of 372 mg of N-carbobenzoxy-D-α-aminovaleric acid *p*-nitrophenyl ester in 17 ml of DMF. The solution was allowed to stand overnight at 37°. After work-up, 307 mg of amorphous powder was obtained. It was dissolved in 25 ml of 50% methanol and hydrogenated over 30 mg of palladium black. After purification on cellulose chromatography, 190 mg of **12** was obtained as white powder: mp 238° (dec.). $[\alpha]_D^{20}$ −4.2° (c=1.000, 1 N HCl). *Anal.* Calcd. for $C_{16}H_{24}N_4O_8 \cdot H_2O$: C, 45.93; H, 6.26; N, 13.39. Found: C, 46.15; H, 5.88; N, 13.51.

5′-N-L-Ornithylpolyoxin C (13)——To a solution of 503 mg of **6** in a mixture of 0.195 ml of triethylamine, 30 ml of water, and 20 ml of DMF, was added a solution of 782 mg of N^{α},N^{δ}-dicarbobenzoxy-L-ornithine *p*-nitrophenyl ester in 25 ml of DMF and the solntion was allowed to stand at 37° for 3 days. After work-up, 610 mg of the product was obtained. A portion of this powder (200 mg) was dissolved in 20 ml of 50% methanol and hydrogenated over 40 mg of palladium black in the presence of a drop of AcOH. After removal of catalyst, 0.3 ml of 1 N HCl was added. The solution was concentrated to a small volume and precipitated by the addition of ethanol, affording 115 mg of **13** hydrochloride. It was purified by cellulose chromatography; mp >200° (dec.). $[\alpha]_D^{20}$ +29.4° (c=1.038, H_2O). *Anal.* Calcd. for $C_{16}H_{25}N_5O_9 \cdot 2HCl \cdot 1/4C_2H_6O$: C, 38.42; H, 5.57; N, 13.58. Found: C, 38.74; H, 5.73; N, 13.78. Solvation of ethanol was detected by NMR spectrum.

5′-N-L-Lysylpolyoxin C (14)——To a solution of 671 mg of **6** in a mixture of 90 ml of 44% aqueous DMF and 0.3 ml of triethylamine, was added a solution of 803 mg of N^{α},N^{ε}-dicarbobenzoxy-L-lysine *p*-nitropheny ester in 25 ml of DMF and the solution was allowed to stand at 37° for 3 days. After work-up 841 mg of powder was obtained; mp 111—117°.

A solution of 201 mg of this powder in 25 ml of 40% methanol was hydrogenated over 30 mg of palladium black in the presence of 2 drops of acetic acid. After work-up as described in the preceding section, 102 mg of **14** (hydrochloride) was obtained as white powder; mp >190° (dec.). $[\alpha]_D^{20}$ +39.4° (c=1.120, H_2O). *Anal.* Calcd. for $C_{17}H_{27}N_5O_9 \cdot 2HCl$: C, 39.38; H, 5.64; N, 13.51. Found: C, 39.51; H, 5.75; N, 13.48.

5′-N-L-Glutamylthyminepolyoxin C (15)——To a solution of 503 mg of **6** in mixture of 30 ml of water, 20 ml of DMF and 172 mg of N-methylmorpholine, were added a solution of 739 mg of γ-benzyl N-carbobenzoxyglutamate *p*-nitrophenyl ester in 30 ml of DMF and the solution was allowed to stand overnight at 37°. After work-up, 400 mg of powder was obtained. 350 mg of this powder was dissolved in 90 ml of 50% methanol and hydrogenated over 35 mg of palladium black, affording 250 mg of **15**. It was purified by cellulose chromatography; mp 193—195° (dec.). $[\alpha]_D^{20}$ +35.8° (c=1.04, H_2O). *Anal.* Calcd. for $C_{16}H_{22}N_4O_{10} \cdot 1/2H_2O$: C, 43.70; H, 5.24; N, 12.75. Found: C, 43.41; H, 5.04; N, 12.60.

5′-N-L-Glutaminylthyminepolyoxin C (16)——To a solution of 615 mg of **6** in a mixture of 30 ml of 33% aqueous DMF and 0.28 ml of triethylamine was added 739 mg of N-carbobenzoxy-L-glutamine *p*-nitrophenyl ester and the solution was allowed to stand for 2 days at room temperature. After work-up, 879 mg of colorless needles were obtained. The crystals (200 mg) were dissolved in 30 ml of water and hydrogenated over 30 mg of palladium black, affording 136 mg of **16** as colorless needles from ethanol, mp 187—189°.

$[\alpha]_D^{20}$ +37.9° (c=1.30, H_2O). *Anal.* Calcd. for $C_{16}H_{23}N_5O_9 \cdot 1/2H_2O \cdot 1/2C_2H_6O$: C, 44.44; H, 5.49; N, 15.25. Found: C, 44.17; H, 5.67; N, 15.48. Solvated ethanol was detected by NMR spectrum.

N^{α}-Carbobenzoxy-N^{δ}-acetyl-L-ornithine *p*-Nitrophenylester——N^{δ}-Acetyl-L-ornithine was perpared by selective acetylation of copper salt of L-ornithine,[17] mp 250° (dec.). Conventional carbobenzoxylation followed by reaction with *p*-nitrophenol and DCC afforded N^{α}-carbobenzoxy-N^{δ}-acetyl-L-ornithine *p*-nitrophenyl ester, mp 152°. $[\alpha]_{578}^{20}$ −25.7° (c=1.14, DMF). *Anal.* Calcd. for $C_{21}H_{23}N_3O_7$: C, 58.73; H, 5.40; N, 9.79. Found: C, 58.59; H, 5.58; N, 9.91.

5′-N-(N^{δ}-Acetyl-L-ornithyl)thyminepolyoxin C (17a) and 5′-N-(N^{δ}-Acetyl-L-ornithyl)polyoxin C (17b)——To a solution of 335 mg of **6** and 0.13 ml of triethylamine in 50% aqueous DMF was added a solution of 427 mg of N^{α}-carbobenzoxy-N^{δ}-acetyl-L-ornithine *p*-nitrophenyl ester and the solution was allowed to stand at 37° for 2 days. After work-up, 450 mg of crystalline product (mp 129—135°) was obtained. A portion (250 mg) was hydrogenated over 70 mg of palladium black in 40 ml of water. After cellulose chromatography, 100 mg of crystalline **17a** was obtained, mp 184—188° (dec.). $[\alpha]_D^{20}$ +33.8° (c=0.551, H_2O). *Anal.* Calcd. for $C_{18}H_{27}N_5O_9 \cdot H_2O$: C, 45.47; H, 6.15; N, 14.73. Found: C, 44.97; H, 6.36; N, 15.13. From the fractions eluted later, 70 mg of **17b** was obtained as colorless powder, mp 180—200° (dec.). $[\alpha]_D^{20}$ +43.8° (c=1.02, H_2O). *Anal.* Calcd. for $C_{18}H_{27}N_5O_{10} \cdot 1/2H_2O$: C, 44.81; H, 5.85; N, 14.52. Found: C, 45.18; H, 5.58; N, 14.29.

5′-N-L-Citrullylthyminepolyoxin C (18)——To a solution of 562 mg of **6** and 0.26 ml of triethylamine in 30 ml of 33% DMF was added 721 mg of N-carbobenzoxy-L-citrulline *p*-nitrophenyl ester in 20 ml of DMF and the solution was stirred for 36 hr at room temperature. After work-up, 930 mg of the product was obtained as colorless crystals, mp 150—159°. A portion (400 mg) was hydrogenated over palladium black (40 mg) in 50 ml of water. After chromatography, 240 mg of crystalline **18** was obtained, mp 190—220°. (dec.) $[\alpha]_D^{20}$ +38.9° (c=1.075, H_2O). *Anal.* Calcd. for $C_{17}H_{26}N_6O_9$: C, 44.54; H, 5.72; N, 18.33. Found: C, 44.28; H, 5.75; N, 18.20.

5′-N-(δ-Carbamoyloxy-DL-α-aminovaleroyl)thyminepolyoxin C (19a) and 5′-N-(δ-Carbamoyloxy-DL-α-aminovaleroyl)polyoxin C (19b)——To a solution of 80 mg of **6** and 0.043 ml of triethylamine in 60% DMF was added 85 mg of **5** and the solution was allowed to react for 3 days at 37°. After work-up, 82 mg of white powder was obtained. A portion (55 mg) was hydrogenated over 5.5 mg of palladium black in 10 ml of 40% methanol. Product was purified by cellulose thin-layer chromatography using butanol–acetic acid-water (4: 1: 2). From the higher *Rf* zone, 15 mg of **19a** was obtained as white powder, mp 185—195° (dec.). *Anal.* Calcd. for $C_{17}H_{25}N_5O_{10} \cdot 1/2H_2O \cdot 1/2C_2H_4O_2$: C, 43.37; H, 5.66; N, 14.05. Found: C, 43.03; H, 5.47; N, 14.05. Solvated acetic acid was indicated by NMR spectrum (δ 2.08, s, 1.5H CH_3COOH). $[\alpha]_D^{20}$ +24.9° (c=1.024, H_2O). From the lower zone, 15 mg of purified powder of **19b** was obtained, mp 180—200° (dec.). *Anal.* Calcd. for $C_{17}H_{25}N_5O_{11} \cdot H_2O \cdot 1/2C_2H_4O_2$: C, 41.29; H, 5.58; N, 13.38. Found: C, 40.96; H, 5.20, N, 13.41. Solvated acetic acid was indicated by NMR spectrum (δ 2.08, s, 1.5H, CH_3COOH).

5′-N-L-Phenylalanylpolyoxin C (20)——To a solution of 335 mg of **6** and 111 mg of N-methylmorpholine in 40% DMF was added a solution of 420 mg of N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester in 14 ml of 50% DMF and the solution was allowed to stand for 15 hr at 37°. After work-up, 283 mg of powder was obtained. A portion (200 mg) was hydrogenated over 20 mg of palladium black in 30 ml of 50% methanol. After chromatography, 54 mg of colorless powder of **20** was obtained, mp 185—190° (dec.). $[\alpha]_D^{20}$ +43.2° (c=1.05, H_2O). *Anal.* Calcd. for $C_{20}H_{24}N_4O_9 \cdot H_2O$: C, 49.79; H, 5.43; N, 11.61. Found: C, 49.80; H, 5.68; N, 11.68.

N^{α}-Carbobenzoxy-N^{δ}-adamantoyl-L-ornithine *p*-Nitrophenyl Ester——To an ice-cooled solution of N^{α}-carbobenzoxy-L-ornithine (2.66 g) in 21 ml of 1 N NaOH, was added 2.2 g of 1-adamantanecarboxylic acid chloride and the solution was allowed to stand overnight at room temperature. The reaction mixture was washed with ether, acidified with 3 N HCl, and extracted with ethyl acetate. It was dried over Na_2SO_4 and concentrated to dryness, affording 3.97 g of colorless foam. A portion (3.47 g) was dissolved in ethyl acetate (20 ml) followed by addition of 1.126 g of *p*-nitrophenol. After cooling in an ice-bath, 1.669 g of DCC was added and the resulting solution was allowed to stand for 1 hr in an ice bath then for a few hr at room temperature. Urea was filtered off and the filtrate was concentrated to dryness. The residue was crystallized from ethanol, mp 57—59°. $[\alpha]_D^{20}$ −10.5° (c=1.00, DMF). *Anal.* Calcd. for $C_{30}H_{35}N_3O_7$: C, 65.56; H, 6.42; N, 7.65. Found: C, 65.99; H, 6.71; N, 7.81.

N^{α}-Carbobenzoxy-N^{δ}-lauroyl-L-ornithine *p*-Nitrophenyl Ester——This compound was prepared in the similar way as in the preceding paragraph, colorless crystals, mp 113—116°. $[\alpha]_D^{20}$ −15.8° (c=1.00, DMF). *Anal.* Calcd. for $C_{31}H_{43}N_3O_7$: C, 65.35; H, 7.61; N, 7.38. Found: C, 65.56; H, 7.45; N, 7.44.

5′-N-(N^{δ}-Adamamtoyl-L-ornithyl)polyoxin C (21)——To a solution of 503 mg of **6** and 0.195 ml of triethylamine in 50 ml of 40% DMF was added 825 mg of N^{α}-carbobenzoxy-N^{δ}-adamantoyl-L-ornithine *p*-nitrophenyl ester in 20 ml DMF. The solution was allowed to stand for 3 days at 37°. After work-up, 700 mg of powder was obtained. It was hydrogenated over 330 mg of 10% palladium on carbon in 40 ml of 66%

18) This compound was prepared in a similar way to the synthesis of N^{ε}-acetyllysine; J.P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 3, John Wiley and Sons, N.Y. 1961, p. 2117.

methanol for 1 hr. The product was purified by cellulose chromatography using butanol–acetic acid–water (7: 1.5: 1), affording 335 mg of purified powder of **21**, mp 200—240° (dec.). $[\alpha]_D^{20}$ +35.7° (c=1.00, 50% methanol). *Anal.* Calcd. for $C_{27}H_{39}N_5O_{10}$: C, 54.63; H, 6.62; N, 11.80. Found: C, 54.22; H, 6.60; N, 11.83.

5′-N-(N$^{\delta}$-Lauroyl-L-ornithyl)polyoxin C (22)——Compound **6** (252 mg) was reacted with 428 mg of N$^{\alpha}$-carbobenzoxy-N$^{\delta}$-lauroyl-L-ornithine p-nitrophenyl ester in the similar way as described in the preceding paragraph. Hydrogenolysis followed by purification afforded 260 mg of purified powder of **22**, mp 185—190° (dec.). $[\alpha]_D^{20}$ +15.3° (c=1.00, DMF). *Anal.* Calcd. for $C_{28}H_{47}N_5O_{10}\cdot 1/2H_2O$: C, 54.00; H, 7.77; N, 11.25. Found: C, 53.73; H, 7.58; N, 11.13.

Glycylpolyoxin L (24)——To a solution of 234 mg of polyoxin L (**23**) and 0.07 ml of triethylamine in 14 ml of 50% DMF was added 165 mg of carbobenzoxyglycine p-nitrophenyl ester. The solution was allowed to stand for 2 days at 37°. The reaction mixture was passed through Dowex 50W X-8 (H^+, 5 ml) and concentrated to a small volume. Product was precipitated with the addition of ethanol–ether, affording white powder (230 mg). It was hydrogenated over 20 mg of palladium black in 10 ml of water for 2 hr. After cellulose chromatography, 110 mg of purified powder of **24** was obtained, mp 193—198°. $[\alpha]_D^{20}$ +7.2° (c=1.07, H_2O). *Anal.* Calcd. for $C_{18}H_{26}N_6O_{13}\cdot 1/2H_2O\cdot 1/2C_2H_6O$: C, 40.28; H, 5.34; N, 14.84. Found: C, 39.90; H, 4.93; N, 14.82. Solvated ethanol was detected by NMR spectrum.

L-Alanylpolyoxin L (25)——Polyoxin L (**23**, 234 mg) was reacted with 172 mg of N-carbobenzoxy-L-alanine p-nitrophenyl ester as described in the preceding paragraph. After hydrogenolysis and purification, 106 mg of purified powder of **25** was obtained, mp 194—200° (dec.). $[\alpha]_D^{20}$ +19.2° (c=1.00, H_2O). *Anal.* Calcd. for $C_{19}H_{28}N_6O_{13}\cdot H_2O$: C, 40.28; H, 5.34; N, 14.84. Found: C, 40.04; H, 5.16; N, 14.33.

D-Alanylpolyoxin L (26)——This compound was prepared in the same way as in the preparation of **25**, affording 140 mg of purified powder **26**, mp 194—200° (dec.). $[\alpha]_D^{20}$ +4.5° (c=1.034, H_2O). *Anal.* Calcd. for $C_{19}H_{28}N_6O_{13}\cdot H_2O$: C, 40.28; H, 5.34; N, 14.84. Found: C, 40.17; H, 5.18; N, 14.54.

L-Leucylpolyoxin L (27)——Compound **23** (234 mg) was reacted similarly with 194 mg of N-carbobenzoxyleucine p-nitrophenyl ester. After hydrogenolysis over 280 mg of 10% palladium on carbon for 45 min and purification by cellulose chromatography using butanol–acetic acid–water (4: 1: 1), 100 mg of purified powder of **27** was obtained, mp 185—190° (dec). $[\alpha]_D^{20}$ +25.9° (c=1.05, H_2O). *Anal.* Calcd. for $C_{22}H_{34}N_6O_{13}\cdot H_2O$: C, 43.42; H, 5.96; N, 13.81. Found: C, 43.76; H, 5.80; N, 13.75.

L-Phenylalanylpolyoxin L (28)——Polyoxin L (**23**, 234 mg) was reacted with 210 mg of N-carbobenzoxy-L-phenylalanine p-nitrophenyl ester. After hydrogenation and chromatography with butanol–acetic acid–water (4: 1: 1.5), 160 mg of **28** was obtained, mp 190—197° (dec.). $[\alpha]_D^{20}$ +29.7° (c=1.04, H_2O). *Anal.* Calcd. for $C_{25}H_{32}N_6O_{13}\cdot 1/2H_2O$: C, 47.39; H, 5.25; N, 13.27. Found: C, 47.27; H, 5.13; N, 13.20.

N$^{\delta}$-Adamantoyl-L-ornithylpolyoxin L (29)——Polyoxin L (**23**, 234 mg) and 0.085 ml of triethylamine in 14 ml of 50% DMF was reacted with 275 mg of N$^{\alpha}$-carbobenzoxy-N$^{\delta}$-adamantoyl-L-ornithine p-nitrophenyl ester in 7 ml of DMF. After work-up, 203 mg of syrup was obtained, which was hydrogenated over 150 mg of palladium on carbon (10%) in 30 ml of 50% methanol for 1 hr. After purification by cellulose chromatography using butanol–acetic acid–water (4: 1: 1), 160 mg of crystalline powder of **29** was obtained, mp 187—192° (dec.). $[\alpha]_D^{20}$ +7.1° (c=1.00, DMF). *Anal.* Calcd. for $C_{32}H_{47}N_7O_{14}\cdot 3/2H_2O$: C, 49.22; H, 6.45; N, 12.56. Found: C, 49.00; H, 6.11; N, 12.70.

N$^{\delta}$-Lauroyl-L-ornithylpolyoxin L (30)——Polyoxin L (**23**, 234 mg) was reacted similarly with 286 mg of N$^{\alpha}$-carbobenzoxy-N$^{\delta}$-lauroyl-L-ornithine p-nitrophenyl ester as in the preceding paragraph. After hydrogenolysis followed by chromatography, 165 mg of purified powder of **30** was obtained, mp 185—188°. $[\alpha]_D^{20}$ +13.0° (c=1.00, DMF). *Anal.* Calcd. for $C_{33}H_{55}N_7O_{14}\cdot 1/2H_2O\cdot 1/2C_2H_6O$: C, 50.41; H, 7.13; N, 11.76. Found: C, 50.63; H, 7.13; N, 11.78. Solvated ethanol was detected by NMR spectrum.

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