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The Structure of Viscosin, A Peptide Antibiotic. II.[1,2] Syntheses of D- and L-3-Hydroxyacylhexapeptides including the Proposed Structure of Viscosin and Its Optical Isomers

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Homologs of D- and L-3-hydroxyacylhexapeptide including compound (I-1c) and its optical isomers (I-1'c, I-2c, I-2'c) were synthesized by condensing the tripeptide subunit with the dipeptide subunit followed by azide coupling of the resulting pentapeptide with D- or L-3-hydroxyacylleucine, and their antibacterial properties were examined. None of the synthetic acylhexapeptides was identical with viscosin in both physical and chemical properties and antituberculous activity.

Based on the proposed structure for viscosin,[4] Hitomi, *et al.*[5] had synthesized six homologs of the acylhexapeptide to test their antituberculous activity and found that myristoyl- and palmitoyl-leucylglycylseryl-D-valylthreonylleucine had a little antituberculous activity.

Recently, we[6] have described the preparation of two diastereoisomeric D-3-hydroxydecanoylhexapeptides, that is, the proposed structure for viscosin (I-1c) and its optical isomer (I-1'c). From their physical and chemical properties and antibacterial activities, it is almost certain that there are doubts on the proposed structure (I-1c).

X–$CH_3(CH_2)_6CH(OH)CH_2CO$–Leu–Gly–Ser–Y–Val–Thr–Leu–OH

	(I-1c)	(I-1'c)	(I-2c)	(I-2'c)
X:	D	D	L	L
Y:	D	L	D	L

In order to clarify structural ambiguities on viscosin we have further carried out the synthesis of the homologs of 3-hydroxyacylhexapeptide including I-1c and its optical isomers (I-1'c, I-2c, I-2'c). We now wish to describe in detail syntheses of the homologs of acylhexapeptide containing C_6—C_{18} 3-hydroxy fatty acids and their antibacterial activities.

For the synthesis of 3-hydroxyacylhexapeptide methyl esters (XI), we used D- or L-3-hydroxyacyl-Leu-$NHNH_2$ (X-1' or X-2'), as the intermediate, prepared in the previous study.[1]

The synthesis was achieved by the route illustrated in Chart 1. Condensation of the tripeptide (VII) with the dipeptide (VI) gave the protected pentapeptide ester (VIII), which was hydrogenated to yield pentapeptide ester (IX). Coupling of IX with acylleucine hydrazide (X-1' or X-2') was performed by the azide method. The protected C-terminal tripeptide methyl ester (V-1 or V-2) was prepared by the following two routes: (i) Z-D(or L)-Val-Thr-OMe (II-1 or II-2) prepared by either the active ester method or the N,N'-dicyclohexylcarbodi-

1) Part I: M. Hiramoto, K. Okada, S. Nagai and H. Kawamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1308 (1971).
2) The abbreviated designations of amino acids, peptides and their derivatives mentioned in this paper are those from *Biochemistry*, **5**, 2585 (1966); **6**, 362 (1967). Amino acid symbols denote the L configuration unless otherwise stated. D-Valine was purchased from Tanabe Amino Acid Research Foundation (Osaka, Japan).
3) Location: *Takara-machi 13, Kanazawa.*
4) T. Ohno, S. Tajima and K. Toki, *Nippon Nogeikagaku Kaishi*, **27**, 665 (1953); K. Toki and T. Ohno, *ibid.*, **29**, 370 (1955).
5) H. Hitomi, M. Uchiyama and K. Fukuda, *Yakugaku Zasshi*, **88**, 299 (1968).
6) M. Hiramoto, K. Okada, S. Nagai and H. Kawamoto, *Biochem. Biophys. Res. Commun.*, **35**, 702 (1969).

imide (DCC) procedure was converted to the corresponding hydrazide (III-1 or III-2) followed by coupling with H-Leu–OMe by means of the azide method. (ii) Z-Thr-Leu-OMe (IV), pre-

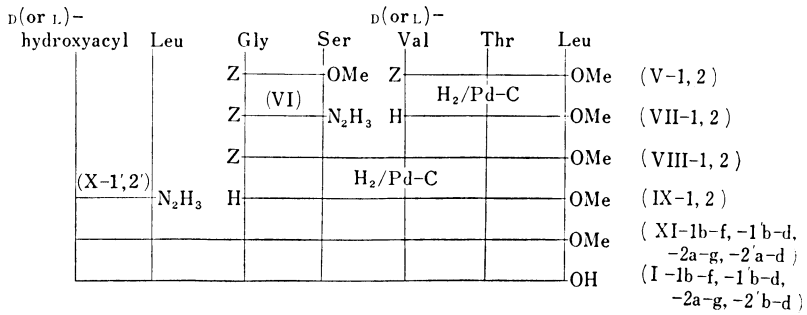

Chart 1. Synthetic Rout to 3-Hydroxyacylhexapeptide

TABLE I. R_1-$CH_3(CH_2)_nCH(OH)CH_2CO$-Leu-Gly-Ser-R_2-Val-Thr-Leu-OMe (XI)

XI-1b-f: $R_1=R_2=$D XI-2a-g: $R_1=$L, $R_2=$D
XI-1'b-d: $R_1=$D, $R_2=$L XI-2'a-d: $R_1=R_2=$L

	n	Yield (%)	mp (decomp. °C)	$[\alpha]_D^{t°}$ (cl, DMF)	TLC Rf_1	TLC Rf_2	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XI-1b[a]	4	48	216.5—218	−22.0[29]	0.93	0.75	$C_{35}H_{64}O_{11}N_6\cdot 2H_2O$	53.83	8.78	10.76	54.24	8.66	10.94
XI-1c[a,b]	6	63	211 —213	−16.0[17]	0.91	0.70	$C_{37}H_{68}O_{11}N_6$	57.49	8.86	10.88	57.50	8.89	10.54
XI-1d[a]	8	75	219 —221	−38.0[26]	0.86	0.72	$C_{39}H_{72}O_{11}N_6\cdot H_2O$	57.19	9.11	10.26	57.45	9.20	10.24
XI-1e[a]	10	78	223.5—225	−13.3[23]	0.95	0.78	$C_{41}H_{76}O_{11}N_6\cdot H_2O$	58.13	9.28	9.92	57.82	9.15	9.63
XI-1f	12	65	219 —223	−17.5[23]	0.95	0.78	$C_{43}H_{80}O_{11}N_6$	59.01	9.44	9.60	59.10	9.52	9.72
XI-1'b	4	48	239 —240	−18.0[29]	0.95	0.48	$C_{35}H_{64}O_{11}N_6\cdot 2H_2O$	53.83	8.78	10.76	53.65	8.61	11.10
XI-1'c[b]	6	42	227.5—229	−12.0[20]	0.73	0.50	$C_{37}H_{68}O_{11}N_6\cdot H_2O$	56.18	8.92	10.62	56.10	8.81	10.53
XI-1'd	8	31	237 —238	−13.0[29]	0.94	0.77	$C_{39}H_{72}O_{11}N_6\cdot H_2O$	57.19	9.11	10.26	56.42	9.04	10.29
XI-2a	2	46	231.5—232.5	−12.0[25]	0.97	0.72	$C_{33}H_{60}O_{11}N_6\cdot H_2O$	53.93	8.50	11.44	54.06	8.50	11.43
XI-2b	4	60	218.5—222.5	−10.0[15]	0.88	0.80	$C_{35}H_{64}O_{11}N_6$	55.10	8.72	11.02	55.11	8.41	10.98
XI-2c[b]	6	65	217.5—220.5	−19.0[17]	0.96	0.79	$C_{37}H_{68}O_{11}N_6$	57.49	8.86	10.88	57.26	8.82	10.63
XI-2d	8	73	225.5—227	−11.8[24]	0.90	0.78	$C_{39}H_{72}O_{11}N_6$	58.48	9.06	10.49	58.07	9.17	10.35
XI-2e	10	73	222 —223.5	− 6.0[24]	0.85	0.79	$C_{41}H_{76}O_{11}N_6\cdot H_2O$	58.13	9.28	9.92	58.63	9.30	10.36
XI-2f	12	73	219.5—220.5	− 9.0[22]	0.93	0.73	$C_{43}H_{80}O_{11}N_6$	59.01	9.44	9.60	59.09	9.38	9.87
XI-2g[a]	14	69	219.5—221	−11.5[23]	0.88	0.54	$C_{45}H_{84}O_{11}N_6$	59.84	9.60	9.31	59.84	9.62	9.01
XI-2'a	2	67	236 —237	−10.0[19]	0.52	0.18	$C_{33}H_{60}O_{11}N_6\cdot H_2O$	53.93	8.50	11.44	54.34	8.68	11.70
XI-2'b	4	52	246.5—247.5	−14.0[23]	0.76	0.44	$C_{35}H_{64}O_{11}N_6$	55.10	8.72	11.02	54.87	9.00	10.70
XI-2'c[b]	6	43	240.5—242.5	− 7.0[20]	0.84	0.59	$C_{37}H_{68}O_{11}N_6\cdot H_2O$	56.18	8.92	10.62	56.20	8.70	10.89
XI-2'd	8	68	247 —247.5	−11.0[17]	0.88	0.45	$C_{39}H_{72}O_{11}N_6\cdot H_2O$	57.19	9.11	10.26	57.10	9.18	9.90

a) The mass spectra of XI-1b, c, d, e, and XI-2g exhibited the expected $(M-H_2O)^+$ peaks at *m/e* 726, 754, 782, 810, and 866, respectively, and also gave the fragment ion peaks characteristic of the amino acid sequences.

b) IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1740 (ester), 1660, 1642 (amide I)

TABLE II. R_1-$CH_3(CH_2)_nCH(OH)CH_2CO$-Leu-Gly-Ser-R_2-Val-Thr-Leu-OH (I)

I-1b-f: R_1=R_2=D I-2a-g: R_1=L, R_2=D
I-1'b-d: R_1=D, R_2=L I-2'b-d: R_1=R_2=L

	n	Yield (%)	mp (decomp °C)	$[\alpha]_D^{25}$ (c 0.5, EtOH)	TLC Rf_1	TLC Rf_2	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
I-1b	4	63	212.5—213.5	−11.0[22]	0.91	0.19	$C_{34}H_{62}O_{11}N_6$	55.87	8.55	11.50	55.57	8.77	11.39
I-1c[a,b,c]	6	85	194 —196	−15.0[15]	0.87	0.20	$C_{36}H_{66}O_{11}N_6$	56.96	8.77	11.07	57.31	8.85	10.81
I-1d	8	85	199.5—201	−18.0[29]	0.83	0.20	$C_{38}H_{70}O_{11}N_6\cdot H_2O$	56.69	9.01	10.44	56.57	9.19	10.34
I-1e	10	43	182.5—184	−13.0[23]	0.88	0.30	$C_{40}H_{74}O_{11}N_6\cdot H_2O$	57.67	9.20	10.09	58.06	9.36	10.50
I-1f	12	84	199.5—201.5	−15.5[21]	0.86	0.20	$C_{42}H_{78}O_{11}N_6\cdot H_2O$	58.58	9.36	9.76	58.95	9.58	10.00
I-1'b	4	57	212.5—214	−24.0[24]	0.80	0.14	$C_{34}H_{62}O_{11}N_6\cdot H_2O$	54.53	8.61	11.22	54.39	8.99	11.02
I-1'c[a,b]	6	84	203.5—206.5	−23.8[15]	0.73	0.16	$C_{36}H_{66}O_{11}N_6\cdot H_2O$	55.65	8.82	10.83	55.83	8.83	10.93
I-1'd	8	89	199 —200	−23.0[24]	0.85	0.19	$C_{38}H_{70}O_{11}N_6\cdot H_2O$	56.69	9.01	10.44	56.99	9.24	10.21
I-2a	2	51	198.5—200.5	−13.5[29]	0.72	0.20	$C_{32}H_{58}O_{11}N_6\cdot H_2O$	53.32	8.39	11.66	52.89	8.43	11.66
I-2b	4	64	196 —198	− 7.5[16]	0.71	0.31	$C_{34}H_{62}O_{11}N_6\cdot H_2O$	54.53	8.61	11.22	54.93	8.77	11.66
I-2c[b]	6	91	198.5—199.5	−12.0[15]	0.81	0.29	$C_{36}H_{66}O_{11}N_6$	56.96	8.77	11.07	56.51	8.74	10.98
I-2d	8	76	201.5—203.5	− 9.0[22]	0.89	0.26	$C_{38}H_{70}O_{11}N_6\cdot H_2O$	56.69	9.01	10.44	56.72	9.21	10.86
I-2e	10	98	205 —206	− 5.0[21]	0.93	0.27	$C_{40}H_{74}O_{11}N_6\cdot H_2O$	57.67	9.20	10.09	57.47	9.30	9.85
I-2f	12	100	200 —201	− 6.0[19]	0.70	0.32	$C_{42}H_{78}O_{11}N_6\cdot H_2O$	58.58	9.36	9.76	58.71	9.51	10.25
I-2g	14	100	195 —197	− 6.0[19]	0.74	0.35	$C_{44}H_{82}O_{11}N_6\cdot H_2O$	59.43	9.52	9.45	59.06	9.44	9.81
I-2'b	4	87	211 —212	−26.5[25]	0.77	0.14	$C_{34}H_{62}O_{11}N_6\cdot H_2O$	54.53	8.61	11.22	54.36	8.86	11.18
I-2'c[a]	6	90	211 —212	−16.4[15]	0.76	0.18	$C_{36}H_{66}O_{11}N_6\cdot H_2O$	55.65	8.82	10.83	55.23	8.81	10.50
I-2'd	8	100	219 —220	−20.0[19]	0.82	0.15	$C_{38}H_{70}O_{11}N_6$	57.99	8.97	10.68	57.42	9.20	10.52

a) Amino acid ratios in acid hydrolysate (105°, 24 hr in 5.7N HCl)
I-1c: Thr, 1.12; Ser, 1.11; Gly, 1.00; Val, 1.11; Leu, 2.19
I-1'c: Thr, 1.1 1; Ser, 1.10; Gly, 1.00; Val, 0.99; Leu, 2.20
I-2'c: Thr, 1.07; Ser, 1.07; Gly, 1.00; Val, 0.98; Leu, 2.20
b) IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1722 (COOH), 1658, 1642 (amide I)
c) pK'=5.9 in 90% MeOH

pared from H-Leu-OMe and Z-Thr-OH by use of the DCC method, was converted to V-1 by interaction with Z-D-Val-ONp. The protected tripeptide methyl ester (V-1 or V-2) was then subjected to catalytic hydrogenolysis and the resulting peptide ester (VII-1 or VII-2) was coupled with Z-Gly-Ser-$NHNH_2$ (VI), which was derived from the corresponding methyl ester,[7] by the azide method to give the protected pentapeptide methyl ester (VIII-1 or VIII-2).

The benzyloxycarbonyl group was removed from the pentapeptide derivative (VIII-1 or VIII-2) by catalytic hydrogenation and the resulting material (IX-1 or IX-2) was coupled with D- or L-3-hydroxyacyl-Leu-$NHNH_2$ (X-1' or X-2') to yield four series of stereoisomeric acylhexapeptide esters (XI–1b-f, –1'b-d, –2a-g and –2'a-d) containing C_6~C_{18} 3-hydroxy-fatty acids. The esters were finally saponified to afford I–1b-f, –1'b-d, –2a-g and –2'b-d.

7) J.I. Harris and J.S. Fruton, *J. Biol. Chem.*, **191**, 143 (1951).

Table III. Antibacterial Activity of 3-Hydroxyacylhexapeptides (I) and Their Methyl Esters (XI)[a]
(Minimum inhibitory concentration, μg/ml)

Compounds	*E. coli*	*St. aureus*	*Candida albicans*
I-1b (XI-1b)	200 (200)	200 (200)	100 (100)
I-1c (XI-1c)	200 (200)	200 (200)	100 (100)
I-1d (XI-1d)	200 (200)	200 (200)	100 (50)
I-1e (XI-1e)	200 (200)	200 (200)	100 (50)
I-1f (XI-1f)	200 (200)	200 (200)	100 (50)
I-1'b (XI-1'b)	200 (200)	200 (200)	100 (50)
I-1'c (XI-1'c)	200 (200)	200 (200)	100 (100)
I-1'd (XI-1'd)	200 (200)	200 (200)	100 (50)
I-2a (XI-2a)	200 (200)	200 (200)	100 (100)
I-2b (XI-2b)	200 (200)	200 (200)	100 (50)
I-2c (XI-2c)	200 (200)	200 (200)	100 (50)
I-2d (XI-2d)	200 (200)	200 (200)	100 (50)
I-2e (XI-2e)	200 (200)	200 (200)	100 (50)
I-2f (XI-2f)	200 (200)	200 (200)	100 (50)
I-2g (XI-2g)	200 (200)	200 (200)	100 (50)
(XI-2'a)	(200)	(200)	(50)
I-2'b (XI-2'b)	200 (200)	200 (200)	100 (50)
I-2'c (XI-2'c)	100 (200)	200 (200)	100 (50)
I-2'd (XI-2'd)	200 (200)	200 (200)	100 (50)
Viscosin	200	200	100
n-Fatty acids[b]	100—200	200	50—100

a) All of the compounds I-1d, e, f, I-1'c, d, I-2a, b, c, e, f, g and I-2'b, c, d showed no activity against *Mycobacterium tuberculosis* even at a concentration of 50 μg per ml of the assay medium, while INAH as a control showed inhibition at a concentration of 0.2 μg per ml. The antituberculous activity of viscosin: 10—20 μg/ml.[8]
b) C_6—C_{18} saturated *n*-fatty acid

The yields, physical constants and elemental analyses of the synthesized compounds (I and XI) are given in Table I and II. The data in Table II obviously showed that neither the melting point nor the $[\alpha]_D$ of any of the synthetic 3-hydroxyacylhexapeptides was identical with those of natural viscosin [mp 270—273°, $[\alpha]_D$-168.3 (c=1, EtOH)].[4]

The two synthetic acylpeptides, I-1c corresponding to the proposed structure of viscosin and its diastereoisomer (I-1'c), were further subjected to chromatographic and amino acid analyses together with infrared (IR) and mass spectrometric examinations. The results obtained revealed the homogeneity of the synthetic compounds and provided clear evidences for discrepancies between the synthetic compound I-1c or I-1'c and viscosin in chemical and physical properties.

The antibacterial activity of the synthetic compounds I and XI was examined toward four microorganisms including tubercle bacillus (Table III). It was found that all of the synthetic compounds tested exhibited only the same or rather less activity as *n*-fatty acids of the corresponding carbon numbers against *E. coli*, *St. aureus* or *Candida albicans*. All our synthetic compounds showed no significant antituberculous activity even at a concentration level of 50 μg/ml that is about five times as high as that of viscosin producing the inhibition.[8] From differences in antituberculous potencies and physical and chemical properties between the synthetic compound I-1c and natural viscosin, it may be concluded that the proposed structure for viscosin should be revised.

Further work on the structure of viscosin is in progress and will be described elsewhere.

8) M. Kochi, V. Groupé, L.H. Pugh and D. Weiss, *Bact. Proc.*, 29, (1951).

Experimental[9]

Thin-layer chromatography was carried out on Wako's silica gel G-5 with the solvent systems of *n*-BuOH–AcOH–H_2O (4:1:5, upper layer, Rf_1) and iso-BuOH–3%NH_3 (3:1, upper layer, Rf_2). The modified Rydon's reagent[10] was used for the detection of the 3-hydroxyacylpeptides. The amino acid composition of the acid hydrolysate was determined with a Hitachi Amino Acid Analyzer, Model KLA-2 according to the direction given by Moore, *et al.*[11] Optical rotations were determined on a Jasco DIP-SL polarimeter. Mass spectra were determined using a Nippon Denshi model JMS-O1SG mass spectrometer.

Z-Val-Thr-OMe (II-2)——i) By *p*-Nitrophenyl Ester Method[12]: II-2 was prepared from Z-Val-ONp (9.3 g, 25.1 mmole) and H-Thr-OMe (3.3 g, 24.7 mmole) according to the method that was used in preparation of Z-D-Val-Thr-OMe by Hitomi, *et al.*[5] Yield, 7.6 g (86%), mp 140—141°, $[\alpha]_D^{20}$ −16.0° (c=1, EtOH). *Anal.* Calcd. for $C_{18}H_{26}O_2N_6$: C, 59.00; H, 7.15; N, 7.64. Found: C, 59.28; H, 7.02; N, 7.74.

ii) By DCC Method[13]: To an ice cooled solution of H-Thr-OMe (1.4 g, 10 mmole) and DCC (2.1 g, 10.2 mmole) in $CHCl_3$ (14 ml) was added portionwise a solution of Z-Val-OH (2.5 g, 10 mmole) in $CHCl_3$ (33 ml). The mixture was allowed to react at 0° for 10 min and at room temperature for additional 6 hr, and then 1 ml of AcOH was added. The precipitated dicyclohexylurea was removed by filtration and the filtrate was washed successively with 0.2 N HCl, 4% $NaHCO_3$ and H_2O. After drying of the organic layer over Na_2SO_4 and evaporation of the solvent, an oily residue obtained was crystallized from ether. Recrystallization from AcOEt–petr. ether gave 2.35 g (64%) of product, mp 139—140°, $[\alpha]_D^{20}$ −17.0° (c=1, EtOH).

Z-D(or L)-Val-Thr-NHNH$_2$ (III-1 or III-2)——II-1 or II-2 was hydrazinolyzed according to Hitomi, *et al.*[5] to give III-1 or III-2. III-1: Yield, 90%, mp 230—231°, $[\alpha]_D^{25}$ −10.0° (c=2.5, 90% AcOH) [lit.[5] mp 234—236°, $[\alpha]_D^{27}$ −10.4° (c=2.5, 90% AcOH)]. III-2: Yield, 95%, mp 231—232.5°, $[\alpha]_D^{19}$ −20.0° (c=2.5, 90% AcOH). *Anal.* Calcd. for $C_{17}H_{26}O_5N_4$: C, 55.72; H, 7.15; N, 15.29. Found: C, 55.73; H, 6.97; N, 15.10.

Z-Thr-Leu-OMe (IV)——Z-Thr-OH (2.51 g, 10 mmole) was mixed with a cold solution of H-Leu-OMe [prepared from the hydrochloride (1.8 g, 10 mmole) and Et_3N (1.4 ml, 0.01 mole)] in CH_2Cl_2 (17 ml). After addition of a solution of DCC (2.2 g, 10 mmole) in CH_2Cl_2 (1.7 ml), the mixture was stirred at room temperature for 3 hr. Dicyclohexylurea was removed by filtration and the filtrate was washed with 0.5 M $NaHCO_3$ and H_2O and dried over Na_2SO_4. Evaporation of the solvent gave an oily residue, which was crystallized from ether–petr. ether: Yield, 2.1 g (56%), mp 60—64°, $[\alpha]_D^{20}$ −29.0° (c=1, EtOH). *Anal.* Calcd. for $C_{19}H_{28}O_6N_2$: C, 59.98; H, 7.42; N, 7.36. Found: C, 59.76; H, 7.33; N, 7.24.

Z-D(or L)-Val-Thr-Leu-OMe (V-1 or V-2)——A) From III: III was coupled with H-Leu-OMe (prepared from the hydrochloride) according to Hitomi, *et al.*[5] to give V-1 or V-2. i) V-1: Yield, 90%, mp 179—180°, $[\alpha]_D^{20}$ −18.5 (c=1.3, DMF); $[\alpha]_D^{19}$ −29.1 (c=0.55, EtOH)[14] [lit.[5] mp 177—178°, $[\alpha]_D^{26}$ −13.5° (c=1.1, EtOH)]. *Anal.* Calcd. for $C_{24}H_{37}O_7N_3$: C, 60.11; H, 7.78; N, 8.76. Found: C, 60.16; H, 7.56; N, 8.88. ii) V-2: Yield, 83%, mp 195—196°, $[\alpha]_D^{19}$ −11.6° (c=1.3, DMF); $[\alpha]_D^{19}$ −40.0° (c=0.55, EtOH). *Anal.* Found: C, 60.41; H, 7.60; N, 8.94.

B) From IV: IV (1.5 g) was hydrogenated over 10% Pd-C in 40 ml of MeOH for 2.5 hr. After filtration and evaporation of the solvent, the residue obtained was dissolved in 10 ml of DMF. To this ice-cooled solution was added Z-D-Val-ONp (1.5 g), and the mixture was stirred at room temperature for 1 hr and then allowed to stand overnight. The mixture was diluted with ice-cooled 1 N NH_4OH (35 ml) and stirred for 30 min. The resulting precipitate was collected by filtration and dissolved in AcOEt (80 ml). The solution was washed successively with H_2O, 4% $NaHCO_3$, H_2O, 2 N AcOH and H_2O and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield a solid residue, which was washed with ether and recrystallized from AcOEt to give V-1: Yield, 1.25 g (65%), mp 176—177°, $[\alpha]_D^{20}$ −27.3° (c=1.1, EtOH).

Z-Gly-Ser-NHNH$_2$ (VI)——To a solution of Z-Gly-Ser-OMe[7] (4.9 g, 15.8 mmole) in MeOH (49 ml) was added hydrazine hydrate (12.1 ml) and the solution was warmed at 50° for 3 hr. After about 20 hr of standing at room temperature, the hydrazide crystallized. The crystalline material was collected by filtration and recrystallized from MeOH: Yield, 4.3 g (89%), mp 214—215°, $[\alpha]_D^{25}$ −8.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{13}H_{18}O_5N_4$: C, 50.32; H, 5.85; N, 18.06. Found: C, 50.50; H, 6.12; N, 17.67.

Z-Gly-Ser-D(or L)-Val-Thr-Leu-OMe (VIII-1 or VIII-2)——a) Amino Component VII-1: V-1 (4.8 g, 10 mmole) was hydrogenated over 10% Pd-C in 300 ml of MeOH containing 10 ml of 1 N AcOH. After filtration, the filtrate was evaporated to dryness *in vacuo*. The oily residue was dissolved in DMF (40 ml) and Et_3N (1.4 ml) was added. The mixture was cooled to −10°.

9) All melting points were uncorrected.
10) R.H. Mazur, B.W. Ellis and P.S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).
11) S. Moore, D.H. Spackmann and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).
12) M. Bodanszky, *Nature*, **175**, 685 (1955).
13) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955); *idem, ibid.*, **78**, 1367 (1956).
14) A higher optical rotation value was also observed for V-1 prepared by the other route as in part B.

b) Z-Gly-Ser-N_3: 4 N $NaNO_2$ (3.04 ml) was added to a solution of Z-Gly-Ser-$NHNH_2$ (VI, 3.1 g, 10 mmole) in 60% AcOH (120 ml) and AcOEt (400 ml) at $-10°$. The mixture was stirred at $-10°$ for 10 min. The AcOEt layer was separated from the aqueous layer, and the latter was again extracted with ice-cooled AcOEt (40 ml × 2). The combined AcOEt solutions were washed successively with ice-cooled saturated NaCl, 4% $NaHCO_3$ solution and ice-H_2O, diluted with cold DMF (80 ml) and dried over $MgSO_4$.

c) Coupling: The azide solution was added to the cold DMF solution of the amino component (VII-1), and the mixture was stirred at $-5°$ for 1 hr and then allowed to stand overnight in a refrigerator to yield a crystalline precipitate. The precipitate was collected by filtration and recrystallized from MeOH to give VIII-1: Yield, 4.2 g (71%), mp 223—224.5°, $[\alpha]_D^{20}$ −20.0° (c=1.3, DMF); $[\alpha]_D^{19}$ −45.4° (c=0.55, MeOH). *Anal.* Calcd. for $C_{29}H_{45}O_{10}N_5$: C, 55.84; H, 7.27; N, 11.23. Found: C, 55.41; H, 6.97; N, 11.42.

In a similar manner, the diastereoisomeric pentapeptide ester (VIII-2) was prepared from VI and V-2; Yield, 74%, mp 245—245.5°, $[\alpha]_D^{20}$ −13.1° (c=1.3, DMF); $[\alpha]_D^{19}$ −56.5° (c=0.55, MeOH). *Anal.* Found: C, 55.65; H, 7.04; N, 11.12.

General Procedure for the Synthesis of D(or L)-3-Hydroxyacyl-Leu-Gly-Ser-D(or L)-Val-Thr-Leu-OH (I)——A. D(or L)-3-Hydroxyacyl-Leu-Gly-Ser-D(or L)-Val-Thr-Leu-OMe (XI): a) Amino Component IX: VIII (1.05 mmole) was hydrogenated over 10% Pd-C in MeOH (350 ml) containing 1 N AcOH (1.5 ml). After filtration, the filtrate was evaporated *in vacuo* and the residue was dissolved in DMF (25 ml). To the solution was added Et_3N (0.14 ml) and the mixture was cooled to $-15°$.

b) Azide of X: 4 N $NaNO_2$ (0.33 ml) was added to a stirred solution of 3-hydroxyacyl-Leu-$NHNH_2$ (X, 1 mmole) in AcOEt (30 ml) containing 60% AcOH (7.5 ml) and 3 N HCl (0.7 ml) at $-10°$, and stirring was continued at $-10°$ for 10 min. Extraction with AcOEt of the azide and the subsequent washings of the extract were carried out in the same manner as in the case of Z-Gly-Ser-N_3. The cold AcOEt solution (60 ml) containing the azide was dried over $MgSO_4$.

c) Coupling: The azide solution was added to the above mentioned cold DMF solution of the amino component (IX), and the mixture was stirred at $-5°$ for 1 hr and left stand in a refrigerator. After further stirring of the mixture at room temperature for 2 hr, the mixture was evaporated under a reduced pressure to remove AcOEt and the residue was poured into 0.1 N AcOH (250 ml). The mixture was further stirred for a few hour and allowed to stand overnight in a refrigerator. The precipitate was collected by filtration and was recrystallized from MeOH or MeOH–ether. The compounds synthesized were listed in Table I.

B. D(or L)-3-Hydroxyacyl-Leu-Gly-Ser-D(or L)-Val-Thr-Leu-OH (I): A solution of XI (0.635 mmole) in DMF (13 ml) was treated with 1 N NaOH (0.77 ml) at room temperature for 1 hr. The solution was diluted with H_2O (70 ml) and filtered. The filtrate was cooled to 0° and then acidified with 1 N AcOH to give a gelatinous material, which was recrystallized twice from 25% aqueous EtOH. The compounds prepared were listed in Table II.

Inhibitory Activity on Microorganisms——The minimum inhibitory concentrations of the compounds were determined by the same procedure as described in the previous paper.[1]

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