Polyoxypeptins A and B Produced by *Streptomyces*: Apoptosis-Inducing Cyclic Depsipeptides Containing the Novel Amino Acid (2*S*,3*R*)-3-Hydroxy-3-methylproline

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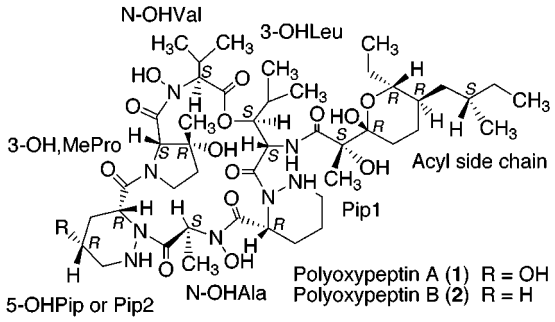
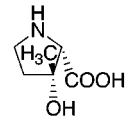
Potent apoptosis-inducing peptides, polyoxypeptins A (**1**) and B (**2**) were isolated from the culture broth of *Streptomyces* sp. by solvent extraction and column chromatography. Structural elucidation of **1** by MS and NMR analyses revealed that it is a cyclic hexadepsipeptide having a novel amino acid and a previously unrecognized *N*-acyl side chain. The depsipeptide consisted of 3-hydroxyleucine, *N*-hydroxyvaline, *N*-hydroxyalanine, piperazic acid, 5-hydroxyhexahydropiperazine-3-carboxylic acid, and an unusual and hitherto unreported amino acid, 3-hydroxy-3-methylproline. Alanine and valine obtained from the hydrolyzate of **1** were both in the L-configuration as concluded from chiral TLC analysis. Then, the absolute structure of **1** was determined with the relative structure obtained from X-ray crystallographic analysis, and the new amino acid was isolated and confirmed to be (2*S*,3*R*)-3-hydroxy-3-methylproline (**3**). MS and NMR of **2** exhibited that it is a monodeoxy compound of **1**. Stereochemistry of **2** was determined by degradation studies. Both **1** and **2** at a concentration of about 0.1 μg/mL induced early cell death, nuclear fragmentation, and internucleosomal DNA scission, all of which are characteristic of apoptosis, in human pancreatic carcinoma AsPC-1 cells.

Introduction

Recently, cell death has been classified into necrosis and apoptosis.[1] The former is accidental cell death, and the latter, programmed cell death induced by cellular signal transduction. Many anticancer drugs such as adriamycin[2] and vinblastine[3] are known to induce apoptosis in cultured cells. However, human solid tumor cells are often resistant to apoptosis when treated with anticancer drugs. We found that human pancreatic adenocarcinoma AsPC-1 cells[4] are an apoptosis-resistant cell line, in which adriamycin and vinblastine inhibit the growth but do not induce apoptosis even at high concentrations. AsPC-1 cells were found to possess highly tyrosine-phosphorylated IGF-1 receptor.[5] Induction of apoptosis would be favorable for anticancer agents, since apoptosis can be induced in a shorter period than that for necrosis. Therefore, we screened microbial cultures for apoptosis inducers effective in AsPC-1 cells. As a result we isolated the novel cyclic hexadepsipeptides polyoxypeptins A (**1**), the subject of a recent brief communication,[6] and B (**2**) from a *Streptomyces* culture broth. They were found to contain a novel amino acid, (2*S*,3*R*)-3-hydroxy-3-methylproline (**3**). In this paper we report the isolation and structural determination of **1** and **2**.





(2*S*,3*R*)-3-Hydroxy-3-methylplorine (**3**)

Results and Discussion

The whole culture broth (5 L) of the polyoxypeptin-producing strain *Streptomyces* MK498-98F14 was extracted with EtOAc, and the extract was then evaporated. The residue (1.36 g) was applied onto a silica gel column and eluted with EtOAc−hexane (5:3) and then $CHCl_3$. First, the minor active compound **2** was eluted and then the major **1**. Recrystallization of **1** with MeOH gave colorless crystals (393.5 mg). Compound **2** was further

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Table 1. ^{1}H and ^{13}C NMR Data for Polyoxypeptin A (1)[a]

no.	position	^{13}C	^{1}H	HMBC	no.	position	^{13}C	^{1}H	HMBC
1	N-OHVal CO	169.4			23	γ	20.7	1.60	
2	α	62.7	5.17	C1, 3, 5, 6	24	δ	46.6	2.82	
								3.14	
3	β	29.4	2.46	C2, 5		δ-NH		4.89	C25
4	γ	19.4	1.04	C2, 3, 5	25	3-OHLeu CO	171.5		
5	γ'	19.7	1.06	C3, 4	26	α	55.9	4.88	C25, 27, 28, 31
	N-OH		8.32		27	β	77.0	5.42	C1, 26, 28, 29, 30
6	3-OH,MePro CO	166.0			28	γ	29.2	1.92	C30
7	α	68.1	4.86	C6, 8, 9, 10, 11	29	δ	15.6	0.96	C27, 28, 30
8	β	78.4			30	δ'	19.9	0.91	C27, 28, 29
9	β-CH_3	27.3	1.47	C7, 8, 10		NH		8.25	C26, 31
10	γ	37.3	1.86	C7, 8	31	acyl chain CO	177.4		
			2.37	C8, 11					
11	δ	45.9	3.24	C10	32	α	76.8		
			4.83	C7, 8, 10					
	β-OH		5.92	C8, 9, 10	33	α-CH_3	20.4	1.37	C31, 32, 34
12	5-OHPip CO	170.7			34	β	99.0		
13	α	47.7	5.43	C12, 14, 15, 17	35	γ	27.8	1.67	C34
								1.74	C34
14	β	29.4	2.02	C12, 15, 16	36	δ	23.9	1.40	
			2.20	C12, 13				1.76	C37
15	γ	59.0	3.65		37	ϵ	35.8	1.27	C38
16	δ	54.3	2.92	C15	38	ζ	38.1	1.02	C36,37,39,40,41,43
			3.06	C15					
	γ-OH		6.90	C14, 15	39	η	31.0	1.40	C38, 40, 41, 42
	δ-NH		4.33	C15	40	η-CH_3	18.6	0.81	C38, 39
17	N-OHAla CO	179.6			41	θ	31.0	1.18	C38, 39, 42
								1.25	C38, 39, 40, 42
18	α	50.9	5.97	C17, 19, 20	42	ι	11.6	0.87	C41
19	β	14.6	1.46	C17, 18	43	ζ'	75.8	3.61	
	N-OH		9.77	C18, 20	44	η'	24.9	1.40	C45
								1.55	C45
20	Pip1 CO	168.2			45	θ'	8.7	0.80	C43, 44
21	α	50.0	5.06	C20, 22, 23		α-OH		3.03	C32, 33, 34
22	β	24.9	1.78	C20, 21		β-OH		6.51	C32, 34, 35
			2.25						

[a] δ (ppm) values were measured in $CDCl_3$ with a JEOL JNM-A500 spectrometer. 3-OH,MePro: 3-hydroxy-3-methylproline, 5-OHPip: 5-hydroxyhexahydropyridazine-3-carboxylic acid, Pip1: piperazic acid.

purified by column chromatography to yield a colorless solid (33.6 mg).

The IR spectrum of **1** exhibited typical absorption bands of amide at 1670 and 1639 cm^{-1} and ester at 1745 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were indicative of a peptide, and it was also suggested by the positive response to the Rydon-Smith reaction.[7] In the positive and negative ion FABMS spectra, the molecular ion peaks of **1** appeared at *m/z* 991 and 967, indicating $(M + Na)^+$ and $(M - H)^-$, respectively. The positive-ion HR-FABMS showed the peak at *m/z* 991.5356 $(M + Na)^+$ ($C_{45}H_{76}N_8O_{15}Na$ requires 991.5328). In FABMS, fragmentation peaks commonly observed for acyclic peptides were not noted. Therefore, a cyclic oligopeptide structure rather than linear was suggested for **1**. An *N*-acylated cyclic structure was also supported by the negative result for the ninhydrin test.

By 2D NMR studies, the structure of **1** was proposed to be a novel cyclic hexadepsipeptide consisting of *N*-hydroxyvaline (N-OHVal), 3-hydroxy-3-methylproline (3-OH,MePro), 5-hydroxyhexahydropyridazine-3-carboxylic acid[8,9] (5-hydroxypiperazic acid, 5-OHPip), *N*-hydroxyalanine (N-OHAla), piperazic acid[8] (Pip1), and 3-hydroxyleucine[10] (3-OHLeu). As shown in Table 1, ^{1}H and ^{13}C chemical shift assignments were made by standard 1D and 2D NMR techniques, such as DEPT, ^{1}H−^{1}H COSY, HMBC, HMQC, HOHAHA, and NOESY. The amino acid sequence and acyl position were determined by HMBC and NOESY. Two NOE's between *N*-OH (δ 9.77) in N-OHAla and α-CH (δ 5.06) in Pip1, and between *N*-OH (δ 8.32) in N-OHVal and α-CH (δ 4.86) in 3-OH,-MePro suggested the presence of two *N*-hydroxyl structures. The structure of the new acyl side chain with 15 carbons was elucidated by conducting ^{1}H−^{1}H COSY, HMBC, and NOESY experiments.

Acid hydrolysis of **1** with 6 N HCl in a sealed tube at 105 °C for 20 h gave several ninhydrin-positive compounds detected by high-voltage paper electrophoresis[11] (HVPE) (3300 V at pH 1.8, R_m, relative mobility to alanine, was measured). A new amino acid (R_m 0.74), (2*S*,3*R*)-3-OH,MePro (**3**) was isolated by resin chromatography on Dowex 50W-X2, followed by preparative HVPE. It was a colorless crystal having mp 197−200 °C; $[\alpha]^{18}_D$ −41° (*c* 0.4, H_2O); $[\alpha]^{23}_D$ −6° (*c* 0.2, 2N HCl). The stereochemistry of **3** was assigned from the X-ray analysis of **1**. L-Ala (R_m 1.0) and L-Val (R_m 0.86) were also isolated from the hydrolysate, and each chirality was determined by application onto an HPTLC plate CHIR developed with MeCN−MeOH−H_2O, 4:1:1.

Since **1** was found to be crystallized from MeOH or MeCN, we performed X-ray crystallographic analysis on

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Table 2. ^{1}H and ^{13}C NMR Data for Polyoxypeptin B (2)[a]

no.	position	^{13}C	^{1}H	HMBC	no.	position	^{13}C	^{1}H	HMBC
1	N-OHVal CO	169.4			23	γ	20.7	1.60	
2	α	62.6	5.19	C1, 3, 4, 5	24	δ	46.7	2.82	
								3.13	C22
3	β	29.4	2.47	C2, 4, 5		δ-NH		4.92	
4	γ	19.5	1.04	C2, 3, 5	25	3-OHLeu CO	171.3		
5	γ'	19.8	1.07	C2, 3, 4	26	α	55.9	4.88	C25, 27, 28, 31
	N-OH		8.32		27	β	77.0	5.41	C1, 26, 28, 30
6	3-OH,MePro CO	166.5			28	γ	29.2	1.91	C29, 30
7	α	67.7	4.83	C6, 8, 9, 10, 11	29	δ	15.6	0.96	C27, 28, 30
8	β	78.5			30	δ'	19.9	0.91	C27, 28, 29
9	β-CH_3	27.4	1.46	C7, 8, 10		NH		8.25	C31
10	γ	37.4	1.83	C7, 8, 9	31	acyl chain CO	177.4		
			2.35	C8, 9, 11					
11	δ	45.0	3.20	C10	32	α	76.8		
			4.71	C10, 12					
	β-OH		5.76	C8, 9	33	α-CH_3	20.4	1.37	C31, 32, 34
12	Pip2 CO	170.4			34	β	99.0		
13	α	46.5	5.28	C12, 14, 15, 17	35	γ	27.8	1.67	C34
								1.74	C34
14	β	23.5	1.89	C12, 13, 15	36	δ	23.9	1.39	
			1.95	C15, 16				1.74	C37
15	γ	19.2	1.38		37	ϵ	35.8	1.25	
			2.47	C14, 16					
16	δ	47.3	2.75		38	ζ	38.1	1.02	C36, 37, 40
			3.13	C14					
	δ-NH		4.18	C15	39	η	31.0	1.40	C38, 40, 41, 42
17	N-OHAla CO	179.3			40	η-CH_3	18.6	0.81	C38, 39, 41
18	α	50.6	5.94	C17, 19	41	θ	31.0	1.19	C38, 40, 42
								1.25	C38, 40, 42
19	β	14.7	1.46	C17, 18	42	ι	11.6	0.87	C39, 41
	N-OH		10.08		43	ζ'	75.8	3.61	C45
20	Pip1 CO	168.2			44	η'	25.0	1.38	C45
								1.56	C45
21	α	49.9	5.07	C20, 22, 23	45	θ'	8.7	0.79	C43, 44
22	β	24.8	1.76	C20, 21		α-OH		3.03	C32, 33, 34
			2.26	C23, 24		β-OH		6.53	C32, 34, 35

[a] δ (ppm) values were measured in $CDCl_3$ with a JEOL JNM-A500 spectrometer. 3-OH,MePro: 3-hydroxy-3-methylproline, Pip1 and Pip2: piperazic acid.

a crystal obtained from the MeCN solution. The absolute structure was not determined by crystallographic analysis, with only relative stereochemistry being corroborated. Since the L-configuration of Ala or Val was determined by hydrolysis of **1**, the absolute structure of the whole compound was elucidated as shown in **1**. The stereochemistry of 5-OHPip was assigned to be (3*R*,5*R*) by the X-ray analysis of **1**. (3*R*,5*R*)-5-OHPip was isolated as a component of himastatin,[9] and the (3*S*,5*S*)-isomer was found in monamycin.[8]

Structurally related compounds of **1**, produced by *Actinomycetes*, include variapeptin,[12] L-156,602,[13] IC101,[14] azinothricin,[15] A83586C,[16] citropeptin,[12] vercopeptin,[17] aurantimycins,[18] and GE3.[19] However, **1** is significantly different from the others, especially in having two new components, **3** and an acyl side chain.

Compound **2** was found to be a deoxy derivative of **1** as judged from the positive and negative ion FABMS spectra, in which the molecular ion peaks appeared at *m/z* 975 and 951, indicating $(M + Na)^+$ and $(M - H)^-$, respectively. Furthermore, HRFABMS of **2** showed the peak at *m/z* 975.5408 $(M + Na)^+$ ($C_{45}H_{76}N_8O_{14}Na$ requires 975.5379). In the ^{1}H NMR of **2** (Table 2), the γ-OH signal (δ 6.90) of 5-OHPip in **1** disappeared. The ^{13}C NMR of **2** showed that β, γ, and δ carbon signals of Pip2 were shifted to higher fields than those of 5-OHPip in **1**. These data indicate that 5-OHPip of **1** is reduced to piperazic acid (Pip2) in **2**. Other NMR data of **2** including the acyl side chain were coincident with those of **1** as shown in Tables 1 and 2. Unlike **1**, we could not prepare satisfactory crystals from **2**; therefore, the stereochemistry was determined by acid hydrolysis of **2**. The chirality of Val or Ala isolated from the hydrolyzate of **2** determined to be L-configuration as the same as the amino acid in **1** on HPTLC plate CHIR. The other three components, 3-OH,-MePro, Pip, and 3-OHLeu, were also identical with those in **1**. The optical rotations of **1** and **2** gave the similar dextrorotatory value with $[\alpha]^{22}_D$ +162° and $[\alpha]^{23}_D$ +147°, respectively. Therefore, the stereochemical structure was deduced as **2**.

Adriamycin and vinblastine even at 30 μg/mL did not induce cell death in 24 h in human pancreatic carcinoma AsPC-1 cells when the viability was assayed by trypan blue dye exclusion. However, they effectively inhibited

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the proliferation of AsPC-1 cells within 2 d, with IC_{50} values of 0.062 and 0.015 μg/mL, respectively. Thus, AsPC-1 is an apoptosis-resistant cell line. Compounds **1** and **2** effectively decreased the viability in AsPC-1 cells with ED_{50} values of 0.08 and 0.17 μg/mL, respectively. Compounds **1** and **2** at 0.1 μg/mL also induced nuclear fragmentation and internucleosomal DNA fragmentation in AsPC-1 cells in 24 h, which are characteristic of apoptosis. For the acute toxicity, the LD_{50} value of **1** in ICR female mice (4 weeks old) was between 63 and 125 μg/mouse by intravenous administration, while that of **2** was 125−250 μg/mouse.

Conclusion

Polyoxypeptins A and B (**1** and **2**) were isolated from a *Streptomyces* culture broth as potent inducers of apoptosis in the apoptosis-resistant cell line AsPC-1. Their cyclic hexadepsipeptide structures contain a simple but hitherto unreported amino acid, (2*S*,3*R*)-3-hydroxy-3-methylproline (**3**).

Experimental Section

^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra at 125 MHz. TLC was performed on a silica gel plate (Kieselgel 60F_{254}, Merck, Art. 5715) which was developed with $CHCl_3$−MeOH, 20:1, and stained with phosphomolybdic acid−H_2SO_4 reagent. The reagent was composed of $Na_2MoO_4{\cdot}2H_2O$ (12 g), 85% H_3PO_4 (7.5 mL), concd H_2SO_4 (25 mL), and H_2O (500 mL). High-voltage paper electrophoresis[10] (HVPE) was carried out on a CAMAG HVE system at 3,300 V for 15 min, with HCOOH−MeCOOH−H_2O (25:75:900, pH 1.8) used as an electrolyte solution, and stained with ninhydrin, red tetrazolium, and Rydon−Smith[7] reagents. The relative mobility (R_m) to alanine was calculated.

Producing Strain. *Streptomyces* strain MK498-98F14 was isolated from a soil sample collected in Aomori Prefecture, Japan, in 1995 by Dr. Masa Hamada, Institute of Microbial Chemistry, and was deposited in the National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tsukuba, Japan, under the accession number FERM P-16200 or FERM BP-6874.

Fermentation. Mycelia of strain MK498-98F14 were inoculated into a 500-mL Erlenmeyer flask containing 110 mL of a medium composed of 2.0% galactose, 2.0% dextrin (Wako Pure Chemical), 1.0% Bacto-Soytone (Difco), 0.5% corn steep liquor (Ajinomoto), 0.2% $(NH_4)_2SO_4$, and 0.2% $CaCO_3$ (adjusted to pH 7.4), and cultured at 27 °C for 3 d on a rotatory shaker at 180 rpm. The seed culture (2.2 mL) was transferred to each of 46 Erlenmeyer flasks containing 110 mL of a medium composed of 1.0% potato starch (Yoshida Seiyaku), 1.0% glucose, 1.0% glycerol, 0.5% Polypeptone (Wako Pure Chemical), 0.5% meat extract (Kyokuto), 0.5% NaCl, and 0.32% $CaCO_3$ (adjusted to pH 7.4). The culture was incubated at 27 °C for 6 d on a rotatory shaker at 180 rpm.

Isolation. The whole culture broth (5 L, pH 7.1) was extracted with an equal volume of EtOAc, and the extract was evaporated to a syrup (1.36 g). The syrup was purified by column chromatography on silica gel (Wako Pure Chemical, C-200, 100 g), which was eluted with EtOAc−hexane (5:3, 1050 mL, fractions 1−78) and then with $CHCl_3$ (250 mL, fractions 79−99).

The major eluate fractions 46−85 were combined and concentrated to yield crystals of **1** (443.2 mg), showing an R_f of 0.59 on TLC. Recrystallization with MeOH gave 393.5 mg of **1**.

The minor eluate fractions 17−26 were combined and concentrated to obtain a crude yellowish solid of **2** (84.9 mg). Further purification by column chromatography on silica gel (8 g), which was eluted with EtOAc−hexane (1:1), gave pure **2** (33.6 mg), which had an R_f of 0.65 on TLC.

Polyoxypeptin A (1): colorless crystals; mp 244−245 °C; $[\alpha]^{22}_D$ +162° (*c* 0.5, $CHCl_3$); UV: λ_{max}^{MeOH} (ϵ) 202 nm (37000), $\lambda_{max}^{0.1\ N\ HCl/MeOH}$ (ϵ) 204 nm (37000), $\lambda_{max}^{0.1\ N\ NaOH/MeOH}$ (ϵ) 210 (101600), 243 nm (sh 11800); IR ν_{max} (KBr): 3381, 3265, 2964, 2935, 2877, 1745 (ester), 1670 (amide), 1639 (amide), 1610, 1506, 1444, 1410, 1302, 1263, 1095, 978, 914 cm^{-1}; FABMS (pos), *m*/*z* 991 $(M + Na)^+$; FABMS (neg), *m*/*z* 967 $(M - H)^-$; HRFABMS (pos), *m*/*z* 991.5356 $(M + Na)^+$ ($C_{45}H_{76}N_8O_{15}Na$ requires 991.5328); ^{1}H and ^{13}C NMR data, see Table 1.

Polyoxypeptin B (2): a colorless solid; mp 157−159 °C; $[\alpha]^{23}_D$ +147° (*c* 0.5, $CHCl_3$); UV: λ_{max}^{MeOH} (ϵ) 202 nm (31000), $\lambda_{max}^{0.1\ N\ HCl/MeOH}$ (ϵ) 205 nm (31000), $\lambda_{max}^{0.1\ N\ NaOH/MeOH}$ (ϵ) 208 (96300), 242 nm (sh 10700); IR ν_{max} (KBr): 3411, 3270, 2962, 2933, 2875, 1743 (ester), 1658 (amide), 1635 (amide), 1506, 1439, 1414, 1311, 1267, 1149, 1097, 1030, 1001, 921 cm^{-1}; FABMS (pos), *m*/*z* 975 $(M + Na)^+$; FABMS (neg), *m*/*z* 951 $(M - H)^-$; HRFABMS (pos.), *m*/*z* 975.5408 $(M + Na)^+$ ($C_{45}H_{76}N_8O_{14}Na$ requires 975.5379); ^{1}H and ^{13}C NMR data, see Table 2.

Acid Hydrolysis of 1. Compound **1** (133.4 mg) was hydrolyzed with 6 N HCl (7 mL) in a sealed tube at 105 °C for 20 h, and the hydrolyzate was diluted with H_2O (50 mL) and washed with diethyl ether (50 mL twice). Evaporation of the aqueous layer containing several ninhydrin-positive compounds (detected by HVPE) gave the residue (114 mg), which was dissolved in 0.1 M pyridine−formate buffer (pH 3.1). The solution was applied onto a column (730 × 16 mm) of Dowex 50W-X2 (100−200 mesh, pyridine form, Dow Chemical, Michigan, 100 mL), which was eluted with the same buffer. Approximately 5.7-mL fractions of the eluate were collected and detected by HVPE.

(2*S*,3*R*)-3-Hydroxy-3-methylproline (3-OH,MePro, 3). The crude solid (20.4 mg) that was obtained from the eluate fractions 28−36 of the Dowex column was subjected to a preparative HVPE (R_m 0.74-band) to obtain (2*S*,3*R*)-3-OH,-MePro (12.1 mg) as colorless crystals: mp 197−200 °C; $[\alpha]^{18}_D$ −41° (*c* 0.4, H_2O); $[\alpha]^{23}_D$ −6° (*c* 0.2, 5 N HCl); FABMS (pos.), *m*/*z* 146 $(M + H)^+$; ^{1}H NMR (D_2O), δ 1.60 (3H, s, CH_3), 2.15 (2H, m, 4-H_2), 3.45 (1H, ddd, 5-Ha), 3.54 (1H, ddd, 5-Hb), 3.86 (1H, s, 2-H); ^{13}C NMR (D_2O), δ 171.2 (C-1), 78.8 (C-3), 70.1 (C-2), 43.7 (C-5), 39.9 (C-4), 24.3 (CH_3).

Chirality of Alanine and Valine. A preparative HVPE (R_m 1.0-band) of the eluate fractions 28−36 gave Ala (1.0 mg): FABMS (pos.), *m*/*z* 90 $(M + H)^+$; ^{1}H NMR (D_2O), δ 1.33 (3H, d, J = 7.4), 3.62 (H, q, J = 7.4). The chirality of Ala was determined to be L by application onto an HPTLC plate CHIR (Merck, Art. 14101, MeCN−MeOH−H_2O 4:1:1). Authentic L-Ala and D-Ala showed R_f 0.41 and 0.37, respectively.

A preparative HVPE (R_m 0.86-band) of the eluate fractions 41−44, followed by a preparative TLC (silica gel, PrOH−H_2O, 7:3, R_f 0.39-band), gave Val (0.9 mg): FABMS (pos.), *m*/*z* 118 $(M + H)^+$; ^{1}H NMR (D_2O), δ 1.0 (3H, d, J = 7.3), 1.05 (3H, d, J = 7.3), 2.28 (1H, dqq, J = 4.4, 7.3 and 7.3), 3.61 (1H, d, J = 4.4). The chirality of Val was also determined to be L by application onto an HPTLC plate CHIR (authentic L-Val: R_f 0.50, D-Val: 0.40).

X-ray Crystallographic Analysis of 1. Compound **1** was crystallized from MeCN solution. Crystal specimen was sealed in a capillary with mother liquor to avoid efflorescence. Crystallographic data: $C_{45}H_{76}N_8O_{15}{\cdot}1.5H_2O$, MW 996.16, orthorhombic, $P2_12_12_1$, a = 17.615 (3), b = 23.547 (3), c = 13.313 (2); V = 5522.0 (14) Å^3, Z = 4, D_x = 1.198 Mg m^{-3}, μ (Mo $K\alpha$) = 0.091 mm^{-1}. The X-ray intensities up to 2θ = 50° were measured on a Rigaku AFC-5 four-circle diffractometer with graphite-monochromatized Mo $K\alpha$ radiation (λ = 0.71073 Å). Final R was 0.124 for 2934 reflections.[20] The relatively large R value may be due to the low resolution of the diffraction data as the result of large thermal motion. Atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the

(20) Edward, C.; Gilmore, C. J.; Mackay, S.; Stewart, N. *CRYSTAN-GM. Version 6.3*, 1996.

Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

Acid Hydrolysis of 2. Compound **2** (278.7 mg) was hydrolyzed with 6 N HCl (7 mL) in a sealed tube at 105 °C for 17 h, and the hydrolyzate was diluted with H_2O (80 mL) and washed with diethyl ether (80 mL twice). Evaporation of the aqueous layer containing several ninhydrin-positive compounds (R_m 1.0, 0.98, 0.86, 0.76, and 0.64 detected by HVPE) gave the residue (236.7 mg), which was dissolved in 0.1M pyridine−formate buffer (pH 2.8). The solution was applied onto a column of Dowex 50W-X2 (200−400 mesh, pyridine form, 300 mL), which was eluted with the same buffer (1200 mL). Approximately 10-mL fractions of the eluate were collected. Evaporation of the eluate fractions 47−52 gave **3** (20.2 mg, R_m 0.76) as colorless crystals ($[\alpha]^{18}_D$ −41°). Concentrations of the eluate fractions 53−55 and fractions 57−60 were obtained L-Ala (3.3 mg, R_m 1.0) and L-Val (2.7 mg, R_m 0.86), respectively. These chiralities were determined by application onto an HPTLC plate CHIR. A mixture (62.0 mg) of Pip (R_m 0.98) and 3-OHLeu (R_m 0.85) obtained from the eluate fractions 74−87 was reacted with 2,4-dinitrofluorobenzene (215 mg) and $NaHCO_3$ (200 mg) in 67% aqueous EtOH at room-temperature overnight. To the reaction mixture, diethyl ether (80 mL), H_2O (80 mL), and 1 N HCl (3 mL) were added, and the ether layer was evaporated to yield the residue (236.7 mg). Two 2,4-dinitrophenyl (DNP) amino acids, DNP-3-OHLeu (9.4 mg) and DNP−Pip (27.9 mg), were isolated by column chromatography on Sephadex LH-20 (Pharmacia AB, developed with MeOH) followed by preparative TLC ($CHCl_3$−MeOH, 5:2). Chiralities of (2*S*,3*S*)-DNP-3-OHLeu, $[\alpha]^{22}_D$ +40° (*c* 0.49, MeOH) and (*R*)-DNP−Pip, $[\alpha]^{23}_D$ +175° (*c* 0.5, MeOH) were determined by comparison with optical rotations of the two DNP-derivatives obtained from **1**.

Cell Viability. AsPC-1 cells were cultured in Roswel Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum, 100 μg/mL kanamycin, 100 units/mL penicillin G, and 30 μg/mL glutamine in a 5% CO_2/95% air atmosphere. Cells (1×10^5) were seeded in 24-well culture plates. After 24 h, chemicals were added to the cells, and then the cells were incubated for 24 h. Cell viability was determined by cell counting with a hemocytometer after staining with trypan blue. Surviving cells were expressed as a percentage of the total cell population. The IC_{50} values are means of triplicate determinations.

Nuclear Fragmentation. Cells (2×10^5) were plated on coverslips in 12-well culture plates and treated with polyoxypeptins for 24 h. Then, the cells were fixed for 15 min with 3% paraformaldehyde solution, and the nuclei were stained for 5 min with the Hoechst 33258 dye. The coverslips were examined under a fluorescence microscope.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1** and **2**, as well as selected 2D NMR spectra, correlation figures, and molecular structure of **1** by X-ray crystallographic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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