Studies on RA Derivatives. V. Synthesis and Antitumor Activity of Ala^2-Modified RA-VII Derivatives[1)]

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A number of RA-VII derivatives having various amino acids including proline (6), pipecolic acid (11), norvaline (12), ornithine (14), aspartic acid (15) and methionine (20) in place of Ala^2 have been synthesized from RA-X methyl ester (3) and evaluated for cytotoxicity to P388 leukemia and KB cells *in vitro*. Comparison of the cytotoxicity of these compounds suggests that the polarity and the length of the 2nd amino acid residue affect the activity. An NMR study revealed that, in solution, 6 and 11 are locked in one conformational state, corresponding to conformer A of RA-VII.

Keywords RA-VII; cyclic hexapeptide; antitumor activity; cytotoxicity; RA-X methyl ester; conformationally locked derivative

RA-VII (**1**) is a bicyclic hexapeptide isolated from the roots of *Rubia akane* and *R. cordifolia* (Rubiaceae).[2)] It has attracted much attention because of its significant antileukemic and antitumor activities coupled with its characteristic structure incorporating the isodityrosine moiety. Recently RA-VII-related compounds which have serine (RA-III, **2**),[2)] threonine (RA-VIII),[3)] pyroglutamic acid (RA-IX) or glutamic acid (RA-X)[4)] in place of alanine at the 2nd position have been isolated from the same plants. The cytotoxicity of these compounds is estimated to range from 0.0023 to 0.37 μg/ml against P388 leukemia cells. These large differences in the activity are presumably due to the changes in the structure at the 2nd position. In order to obtain information about the structural effect of the 2nd amino acid (Aa^2) side chain on the cytotoxic activity, we planned to prepare a number of Aa^2-modified RA derivatives which have not been isolated from natural sources.

This paper describes the preparation of Ala^2-modified RA-VII derivatives, some aspects of their conformations in solution, and the evaluation of their antitumor activity.

Chemistry The starting material was RA-X methyl ester (**3**).[4)] The manipulation of the ester group of **3** afforded proline (**6**), pipecolic acid (**11**), norvaline (**12**), ornithine (**14**), aspartic acid (**15**), homoserine (**17**), vinyl glycine (**19**) and methionine (**20**) derivatives. The synthetic scheme for these derivatives is depicted in Charts 1 and 2.

RA-X methyl ester (**3**) was reduced with lithium borohydride ($LiBH_4$) in tetrahydrofuran (THF) at room temperature to provide the alcohol **4** in 74% yield. The alcohol **4** was treated with methanesulfonyl chloride (MsCl) and triethylamine (Et_3N) at $-78\,^{\circ}C$ in dichloromethane (CH_2Cl_2) in the presence of 4-dimethylaminopyridine (DMAP) to afford the mesylate **5** in 96% yield. Compound **5** was then cyclized in CH_2Cl_2–50% NaOH containing 0.5 eq of tetra-*n*-butylammonium bromide (*n*-Bu_4NBr) to provide the proline derivative **6** in 82% yield.

Preparation of the pipecolic acid derivative **11** required C_1 elongation, which was conducted by means of the following procedures. The mesylate **5** was reacted with *o*-nitrophenyl selenocyanate (*o*-$NO_2PhSeCN$) and sodium borohydride ($NaBH_4$)[5)] in EtOH, and hydrogen peroxide oxidation of the resultant aryl selenide provided the allylglycine derivative **7** in 94% yield. Compound **7** was subjected to Lemieux–Jonson oxidation with osmium tetroxide (OsO_4) and sodium periodate ($NaIO_4$) in dioxane–H_2O to provide the aldehyde **8** in 76% yield. Condensation of **8** with ethyl diethylphosphonoacetate and sodium hydride (NaH) in the presence of 18-crown-6 in THF gave α,β-unsaturated ester **9** (72%), which was subjected to catalytic hydrogenation, and successive reduction of the ester group with $LiBH_4$ provided the alcohol **10** in 31% yield. The alcohol **10** was converted to the pipecolic acid derivative **11** *via* the mesylate in a manner similar to that described for **6** in 56% yield from **10**.

Catalytic hydrogenation of **7** afforded the norvaline derivative **12** in 87% yield. The ornithine derivative **14** was obtained by triphenylphosphine (Ph_3P) reduction of the azide intermediate **13**, which was prepared from the mesylate **5**.

Aspartic acid (**15**), vinylglycine (**19**) and methionine (**20**) derivatives were prepared from the aldehyde **8**. Direct oxidation of the aldehyde **8** with silver(II) oxide (AgO) in THF–H_2O afforded the aspartic acid derivative **15** in poor yield (14%). However, more satisfactory results were obtained by a two-step procedure involving hemiacetal oxidation followed by saponification of the resultant ester. Thus, the aldehyde **8** was reacted with *N*-iodosuccinimide (NIS) in methanol (MeOH)[6)] to afford the methyl ester **16**, which was successively treated with lithium hydroxide (LiOH) in MeOH–H_2O to provide **15** in 59% yield from **8**.

The aldehyde **8** was reduced with $NaBH_4$ in 1,4-dioxane, and the resulting alcohol **17** (90%) was mesylated to afford **18** in 71% yield. The vinylglycine derivative **19** was prepared from **18** in the same manner as described for **7** in 17% yield. Substitution reaction of **18** with sodium thiomethoxide ($NaSCH_3$) in chloroform ($CHCl_3$) or THF gave the oxazine **21** as a major product with a small amount of the desired methionine derivative **20**. However, after conversion of **18** into the corresponding iodide, treatment with $NaSCH_3$ in MeOH provided **20** in 91% yield.

All stereogenic centers of the obtained derivatives remained intact through the reactions, as indicated by comparisons of their chemical shifts and/or coupling constants in the proton (^1H) and carbon 13 (^{13}C) nuclear magnetic resonance (NMR) spectra with those of RA-VII (Table I).

Conformational Information Naturally occurring RAs

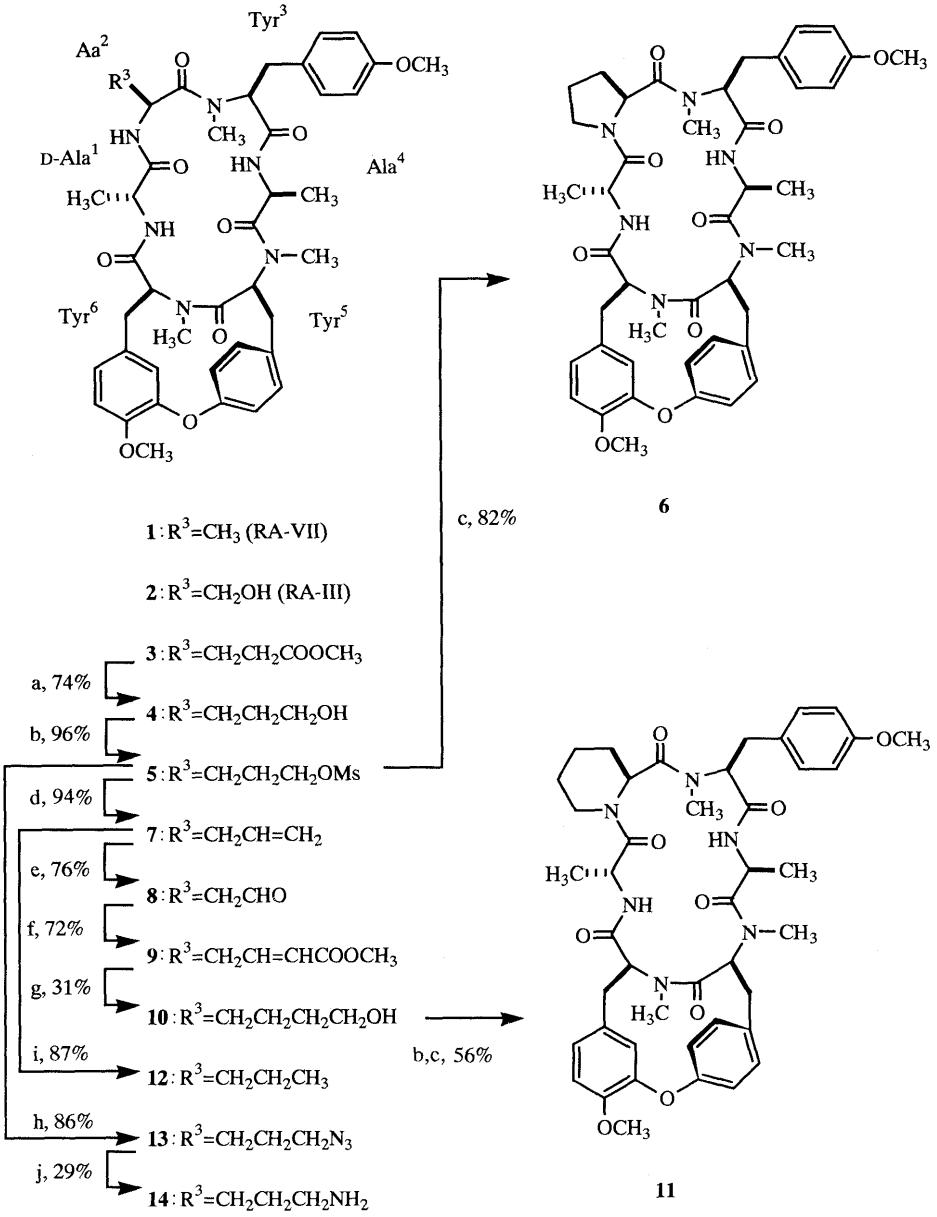

Chart 1. Structure and Synthetic Scheme of RA Derivatives

Ala = L-alanine, Tyr = *N*,*O*-dimethyl-L-tyrosine, D-Ala = D-alanine, Aa = L-amino acid. a) $LiBH_4$, THF, r.t., 12 h. b) MsCl, DMAP, Et_3N, CH_2Cl_2, −78 °C, 1 h. c) *n*-Bu_4Br, CH_2Cl_2–50% NaOH, r.t., 1 h. d) *o*-$NO_2PhSeCN$, $NaBH_4$, EtOH, r.t., 3 h; H_2O_2, THF–EtOH, r.t., 12 h; $(CH_3)_2S$, r.t., 12 h. e) OsO_4, $NaIO_4$, 1,4-dioxane–H_2O, r.t., 18 h. f) Ethyl diethylphosphonoacetate, NaH, 18-crown-6, THF, r.t., 12 h. g) H_2, 10% Pd–C, 1,4-dioxane, r.t., 24 h; $LiBH_4$, THF, r.t., 24 h. h) NaN_3, 18-crown-6, CH_2Cl_2, reflux, 9 h. i) H_2, 10% Pd–C, 1,4-dioxane, r.t., 24 h. j) Ph_3P, H_2O, THF, r.t., 62 h.

adopt two or three conformational states in solution. ^{1}H- and ^{13}C-NMR spectroscopy, including two-dimensional (2D) NMR and molecular dynamics calculation studies for RA-VII revealed the two conformers, A and B, observed in $CDCl_3$ solution to be the *trans* and *cis* amide isomers with respect to the peptide bond between Ala^2 and Tyr^3, respectively.[7] The conformer ratio of natural RAs is influenced by structure, solvent and temperature, and the conformer A predominates (*ca.* 80—100%) in all cases. Similar conformational tendencies were observed in the derivatives prepared in this study. Among them, the proline (**6**) and pipecolic acid (**11**) derivatives showed a single conformer in various solvent(s), which suggested that these compounds are conformationally locked in one rigid structure (Table II). To elucidate the solution conformation of **6**, conformational analysis was performed.

All proton and carbon resonances of **6** were unambiguously assigned by ^{1}H–^{1}H correlated spectroscopy (H–H COSY), [8] heteronuclear multiple quantum coherence (HMQC)[9] and heteronuclear multiple bond connectivity (HMBC)[6] spectra. The ^{1}H- and ^{13}C-NMR parameters of **6** are quite similar to those of the major conformer of RA-VII. Phase-sensitive nuclear Overhauser effect spectroscopy (NOESYPH)[10] correlations between Pro^2-C_αH and Tyr^3-NMe; Tyr^3-NMe and Tyr^3-C_αH also suggest the presence of a *trans* peptide bond between Pro^2 and Tyr^3 (Fig. 1). The temperature coefficients ($\Delta\delta/\Delta T$) of the Ala^4-NH shifts in dimethyl sulfoxide (DMSO)-d_6[11] indicate internal hydrogen bonding to D-Ala^1 carbonyl oxygen, which can stabilize the backbone conformation (Table III).

Circular dichroism (CD) curve of **6** shows positive Cotton

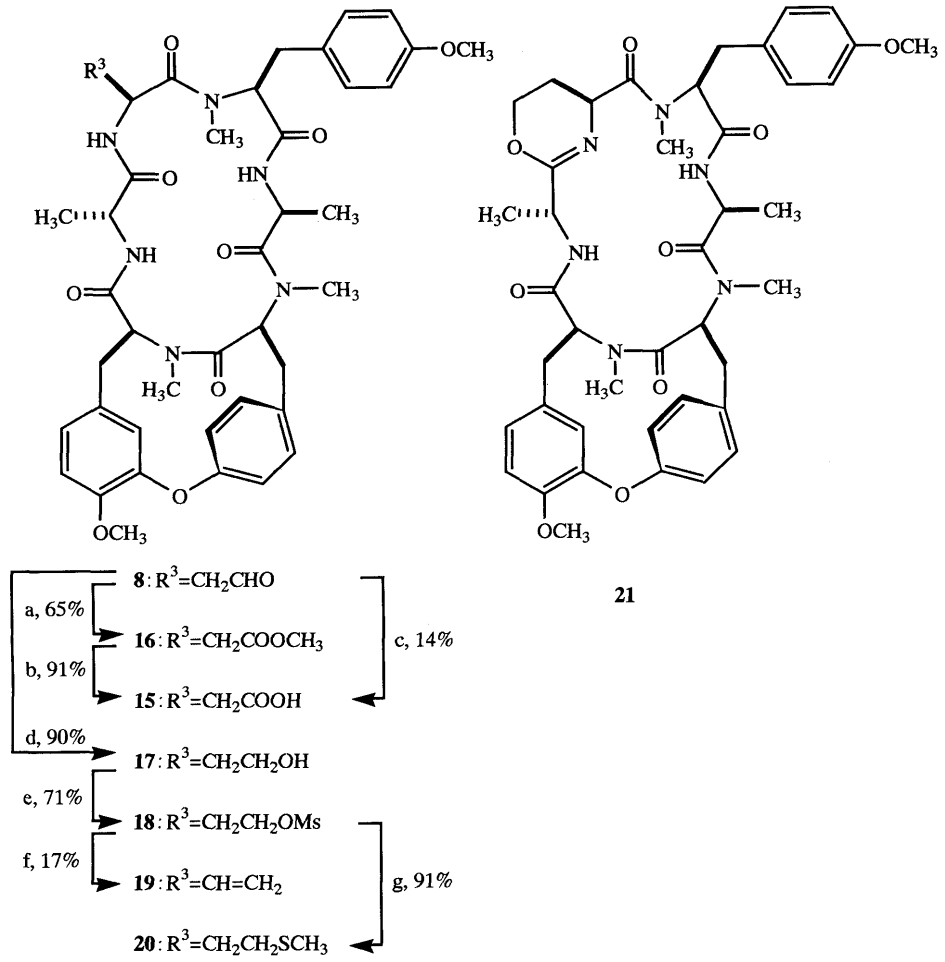

Chart 2

a) NIS, K_2CO_3, CH_3OH, r.t., 7 h. b) LiOH, CH_3OH : H_2O = 2 : 1, r.t., 4 h. c) AgO, THF : H_2O = 9 : 1, reflux, 9 h. d) $NaBH_4$, 1,4-dioxane, r.t., 3 h. e) MsCl, DMAP, Et_3N, CH_2Cl_2, −78 °C, 2 h. f) *o*-$NO_2PhSeCN$, $NaBH_4$, EtOH, r.t., 24 h; H_2O_2, THF–EtOH, r.t., 12 h; $(CH_3)_2S$, r.t., 2 h. g) NaI, $CH_3COCH_2CH_3$, reflux, 10 h; $NaSCH_3$, CH_3OH, r.t., 0.5 h.

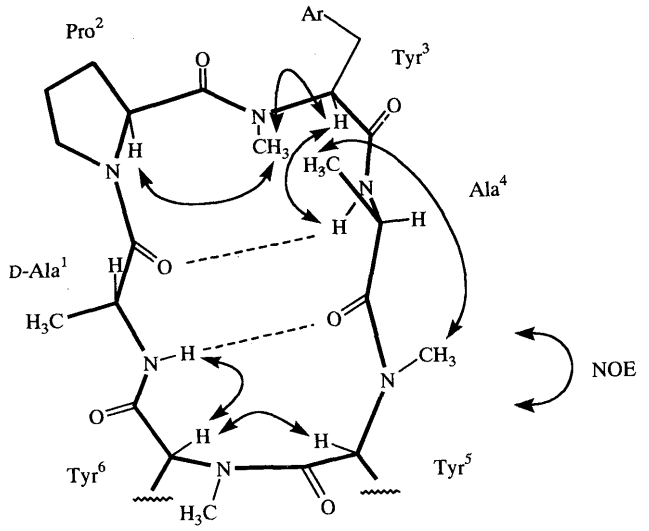

Fig. 1. Selected NOE Correlations of **6** in DMSO-d_6 at 303 K

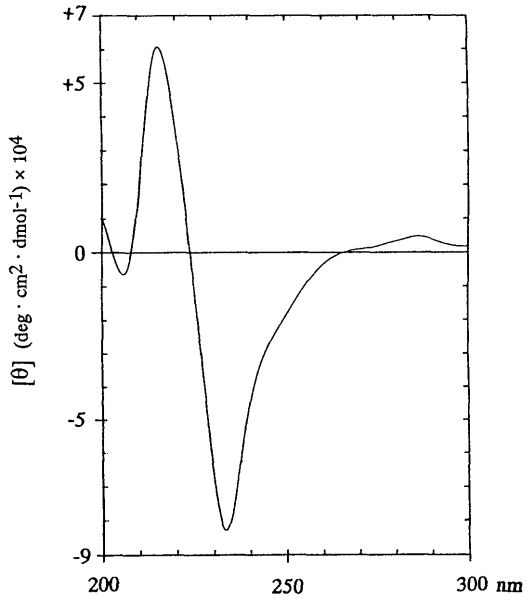

Fig. 2. The CD Spectrum of **6** in CH_3OH

effects at 287 and 215 nm, and negative ones at 233 and 206 nm. The strong band indicates rigid backbone geometry (Fig. 2).

Biological Results and Discussion Thirteen RA derivatives including intermediates prepared in this study were evaluated for *in vitro* cytotoxicity against P388 leukemia and KB cells, and RA-VII (**1**) and III (**2**) were re-evaluated for comparison (Table IV).

Compounds which possess a polar functionality at the side chain showed reduced activity, especially in the case

TABLE I. ^{13}C-NMR Spectral Data for RA Derivatives

Amino acid carbon		4	6	7	8	9	10	11	12	13	14	15[a]	16	17	19	20
D-Ala[1]	C_α	47.93	46.93	47.96	46.18	47.59	48.00	46.23	47.90	48.06	47.81	46.31	46.23	46.93	47.94	47.79
	C_β	20.70	18.17	20.87	21.05	20.94	20.84	18.18	20.84	20.94	20.89	20.79	20.81	20.82	20.70	20.85
Aa[2]	C_α	48.94	55.51	48.20	47.95	47.94	49.06	47.84	48.67	48.41	49.25	47.79	47.92	48.09	51.88	47.82
	C_β	27.76	25.26	35.50	45.87	32.79	30.96	17.97	33.33	25.02	23.68	35.25	35.93	33.47	131.52	30.40
	C_γ	28.26	28.16	132.06	197.96	142.40	22.04	24.46	18.84	28.48	27.35		52.08	58.70	120.16	30.05
	C_δ	61.94	46.78	119.58		125.49	32.11	25.11	13.80	50.84	39.36					15.36
	C_ε					60.60	62.17	42.75								
	C_ζ					14.22										
Tyr[3]	C_α	68.41	68.28	68.69	69.21	68.84	68.51	67.96	68.56	68.52	68.62	68.92	68.95	68.58	68.57	68.38
	C_β	32.99	32.96	32.92	32.75	32.79	32.97	32.89	32.92	32.92	32.96	32.63	32.82	32.97	32.75	32.84
	C_γ	130.67	131.03	130.86	130.93	130.70	130.78	130.21	130.81	130.61	130.98	130.47	131.03	130.78	130.61	130.57
	C_δ	130.14	130.20	130.29	130.29	130.12	130.25	130.21	130.21	130.19	130.25	130.22	130.27	130.13	130.32	130.09
	C_ε	114.12	114.03	114.05	114.06	114.18	114.08	114.02	114.00	114.11	114.31	114.10	114.06	114.09	114.04	114.00
	C_ζ	158.43	158.30	158.42	158.36	158.45	158.43	158.38	158.39	158.27	158.25	158.32	158.29	158.44	158.43	158.09
	C_N	39.95	39.91	39.84	40.25	33.93	39.91	39.46	39.81	39.88	40.16	40.21	40.09	39.86	39.68	39.98
	C_O	55.25	55.24	55.28	55.26	55.23	55.29	55.23	55.23	55.28	55.23	55.23	55.23	55.29	55.25	55.20
Ala[4]	C_α	46.36	46.27	46.37	43.97	46.30	46.39	45.58	46.32	46.39	46.23	45.52	45.37	46.47	46.51	46.22
	C_β	18.54	18.15	18.60	16.80	18.60	18.60	17.97	18.50	18.58	18.67	18.44	18.67	18.51	18.41	18.51
Tyr[5]	C_α	54.28	54.86	54.22	55.26	54.19	54.22	54.69	54.19	54.13	54.59	54.26	54.31	54.11	54.21	54.15
	C_β	36.98	36.82	37.04	37.00	37.02	37.03	36.87	36.97	37.05	36.99	36.90	36.99	36.97	36.99	36.91
	C_γ	135.08	135.72	135.11	135.06	135.05	135.09	135.52	135.09	135.03	135.33	134.92	135.12	134.99	135.10	135.06
	$C_{\delta a}$	132.77	132.66	132.79	132.73	132.77	132.78	132.69	132.75	132.81	132.69	132.72	132.72	132.91	132.81	132.67
	$C_{\delta b}$	130.93	130.87	131.00	130.97	130.96	131.00	130.93	130.96	131.01	130.98	130.93	130.94	130.91	130.96	130.95
	$C_{\varepsilon a}$	124.22	124.04	124.27	124.27	124.28	124.29	124.06	124.21	124.31	124.20	124.22	124.23	124.32	124.26	124.15
	$C_{\varepsilon b}$	125.88	125.91	125.92	125.95	125.91	125.93	125.89	125.89	125.93	125.95	125.94	125.91	125.90	125.91	125.85
	C_ζ	158.24	158.18	158.26	158.23	158.25	158.27	158.17	158.23	158.50	158.56	158.17	158.37	158.31	158.24	158.31
	C_N	30.51	30.66	30.50	30.47	30.47	30.52	30.52	30.46	30.50	30.58	30.48	30.42	30.56	30.53	30.45
Tyr[6]	C_α	57.41	56.98	57.44	57.45	57.45	57.45	57.11	57.41	57.47	57.37	57.37	57.49	57.45	57.41	57.30
	C_β	35.44	35.75	35.40	35.29	35.33	35.40	35.72	35.38	35.35	35.46	35.60	35.36	35.30	35.46	35.31
	C_γ	128.18	128.31	128.15	128.05	128.09	128.15	128.29	128.19	128.10	128.34	128.01	128.23	128.10	128.17	128.02
	$C_{\delta a}$	120.67	121.16	120.91	120.90	120.90	120.92	121.04	120.89	120.89	121.06	120.90	120.90	120.94	120.92	120.84
	$C_{\delta b}$	113.45	113.34	113.44	113.38	113.41	113.43	113.37	113.44	113.42	113.43	113.32	113.54	113.43	113.43	113.28
	$C_{\varepsilon a}$	112.40	112.32	112.35	112.31	112.33	112.35	112.35	112.38	112.32	112.41	112.21	112.55	112.35	112.34	112.18
	$C_{\varepsilon b}$	153.15	153.12	153.15	153.10	153.12	153.15	153.12	153.13	153.14	153.13	152.98	153.22	153.14	153.12	152.98
	C_ζ	146.52	146.57	146.55	146.51	146.52	146.55	146.55	146.51	146.54	146.55	146.42	146.58	146.55	146.52	146.38
	C_N	29.28	29.61	29.24	29.21	29.20	29.26	29.51	29.21	28.48	29.37	29.27	29.19	29.23	29.28	29.18
	C_O	56.17	56.15	56.19	56.15	56.16	56.18	56.15	56.17	56.18	56.20	56.09	56.23	56.13	56.18	56.07
$C_{C=O}$		168.02	168.59 (Tyr[3])	167.80	167.73	165.63	167.90	168.52	167.69	167.71	168.03	167.94	167.78	167.87	167.87	167.82
		169.36	168.67 (Tyr[5])	169.42	169.50	167.57	169.43	168.78	169.40	169.48	169.16	169.40	169.47	169.46	169.37	169.31
		170.68	170.08 (D-Ala[1])	170.62	170.70	169.45	170.65	170.69	170.60	170.54	170.56	170.78	170.28	170.50	170.42	170.53
		171.75	170.94 (Tyr[6])	171.36	170.78	170.56	171.77	171.67	171.73	171.62	171.43	171.21	170.68	171.83	170.62	171.40
		172.11	171.71 (Ala[4])	171.77	171.61	170.78	172.08	171.78	172.14	171.76	172.17	171.44	170.75	171.89	171.79	171.57
		172.33	172.01 (Pro[2])	172.07	171.97	171.67	172.28	172.81	172.24	172.51	172.93	172.12	171.67	172.55	172.19	172.31
						172.33						172.21	171.95			

In $CDCl_3$, major conformer, 100 MHz, δ-values. *a*) 75 MHz.

TABLE II. Conformer Ratio (A : B) of RA Derivatives

Compound	$CDCl_3$		DMSO-d_6		CD_3OD	
	A	B	A	B	A	B
RA-VII[6]	89 :	11				
6	100 :	0	100 :	0	100 :	0
11	100 :	0				

TABLE III. Effect of Temperature on the NH Chemical Shift of **6** in DMSO-d_6

	D-Ala[1]	Ala[4]
$\Delta\delta/\Delta T$ (ppm/K)	4.66×10^{-3}	-3.16×10^{-3}

of ornithine (**14**) and aspartic acid (**15**) derivatives. In this connection, it is noteworthy that ε-hydroxynorleucine (**10**) and homoserine (**17**) derivatives are less toxic than

TABLE IV. Cytotoxicity of RA Derivatives toward P388 Leukemia and KB Cells

Compound	IC_{50} (μg/ml)	
	P388	KB
1 (RA-VII)	0.0013	0.0023
2 (RA-III)	0.011	0.024
4	0.14	0.084
6	0.079	0.074
7	0.031	0.059
8	0.027	0.031
10	0.18	0.14
11	0.079	0.21
12	0.011	0.019
13	0.030	0.013
14	>10	>10
15	0.99	1.88
17	0.048	0.052
19	0.020	0.042
20	0.017	0.040

TABLE V. *In Vivo* Antitumor Activity of RA Derivatives toward P388 Leukemia

Compound	T/C (%) Dose (mg/kg)					
	0.4	1.6	3.13	6.25	12.5	25.0
1 (RA-VII)	144	152	163	Toxic		
2 (RA-III)	149	156		160	159	
6	101	107		121	128	132
15	96	103		126		
20	116	146		158		

P388 cells (10^6) were transplanted intraperitoneally (i.p.) into CDF_1 mice on day 0 and the compound were administered i.p. on days 1—5. T/C (%) = (mean survival time of tested mice)/(mean survival time of control mice) × 100.

methionine (**20**) and norvaline (**12**) derivatives, respectively, having a non-polar residue with similar length.

Another clear relationship exists between the length of the residue and cytotoxicity. In a series of compounds having a hydroxyl group at the end of the side chain, cytotoxicity decreases with increase in the length of the carbon chain (**2** > **17** > **4** > **10**). The same tendencies are observed among other homologues (*e.g.* **19** > **7** : **1** > **12**). However, the observation that the azido intermediate **13** having a rather long residue shows high cytotoxicity suggests that a lengthy side chain can be compatible with activity in the case of a less polar functionality.

The conformationally locked derivatives, **6** and **11**, show potent cytotoxicity, suggesting that this conformation is an active one for expressing the activity.

The proline (**6**), aspartic acid (**15**) and methionine (**20**) derivatives were evaluated for *in vivo* antitumor activity against P388 leukemia (Table V). The observed antitumor activities of these compounds approximately paralleled the cytotoxicities.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and were not corrected. Infrared (IR) spectra were taken on a Perkin Elmer 1710 spectrophotometer. Optical rotations were measured with a JASCO DIP-360 polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AM-400, AM-500 and Varian Gemini 300 spectrometers. Chemical shifts were expressed in ppm with tetramethylsilane as an internal standard. The mass spectra (MS) were taken with Hitachi M-80 and VG AutoSpecE spectrometers. The ultraviolet (UV) and visible absorption spectra were recorded on a Shimadzu UV-240 spectrometer. CD spectra were recorded on a JASCO J-702 spectrometer. Silicagel column chromatography was performed with a CIG column system (22 mm i.d. × 100 mm, Kusano Scientific Co., Tokyo) prepacked with 10 μ silica gel.

[2-(δ-Hydroxynorvaline)]-RA-VII (4) A solution of $LiBH_4$ in THF (2 M, 2.0 ml, 4.0 mmol) was added to a solution of **3** (842.4 mg, 1.0 mmol) in THF (30 ml). The mixture was stirred at room temperature for 12 h, then concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **4** (603 mg, 74%) as a colorless powder, mp 231—232 °C, $[\alpha]_D$ −93.3° (c = 0.15, $CHCl_3$). IR ν ($CHCl_3$): 3404, 1673, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 220 (4.48), 278 (3.63), 283 (3.59). High-resolution FAB-MS Calcd for $C_{43}H_{55}N_6O_9$: 815.3980 $[M+H]^+$, Found: 815.3915. FAB-MS m/z (%): 815 (10, $[M+1]^+$), 121 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.10 (3H, d, J = 6.7 Hz, Ala^4-H$_\beta$), 1.30 (3H, d, J = 6.8 Hz, Ala^1-H$_\beta$), 1.55—1.61 (2H, m, Nva^2-H$_\beta$), 1.79—1.88 (2H, m, Nva^2-H$_\gamma$), 2.63 (1H, dd, J = 11.4, 3.0 Hz, Tyr^5-H$_{\beta a}$), 2.68 (3H, s, Tyr^6-NMe), 2.93 (3H, s, Tyr^3-NMe), 2.99 (1H, dd, J = 18.3, 4.2 Hz, Tyr^6-H$_{\beta a}$), 3.09 (1H, dd, J = 18.3, 12.1 Hz, Tyr^6-H$_{\beta b}$), 3.11 (3H, s, Tyr^5-NMe), 3.27 (1H, dd, J = 14.5, 11.3 Hz, Tyr^3-H$_{\beta a}$), 3.40 (1H, dd, J = 14.5, 4.0 Hz, Tyr^3-H$_{\beta b}$), 3.61 (1H, dd, J = 11.3, 4.0 Hz, Tyr^3-H$_\alpha$), 3.63—3.69 (3H, m, Nva^2-H$_\delta$), 3.78 (3H, s, Tyr^3-OMe), 3.92 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J = 1.8 Hz, Try^6-H$_{\delta a}$), 4.41 (1H, dq, J = 7.1, 6.8 Hz, Ala^1-H$_\alpha$), 4.55 (1H, dd, J = 12.0, 4.2 Hz, Tyr^6-H$_\alpha$), 4.71—4.76 (2H, m, Ala^1-H$_\alpha$ and Nva^2-H$_\alpha$), 5.40 (1H, dd, J = 11.4, 3.0 Hz, Tyr^5-H$_\alpha$), 6.54 (1H, d, J = 7.1 Hz, Ala^1-NH), 6.57 (1H, dd, J = 8.4, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.57 (1H, dd, J = 8.4, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.74 (1H, d, J = 7.6 Hz, Ala^4-NH), 6.79 (1H, d, J = 8.4 Hz, Tyr^6-H$_\varepsilon$), 6.82 (2H, d, J = 8.6 Hz, Tyr^3-H$_\varepsilon$), 6.87 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.05 (2H, d, J = 8.6 Hz, Tyr^6-H$_\delta$), 7.19 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J = 8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.40 (1H, dd, J = 8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

[2-(δ-Hydroxynorvaline)]-RA-VII Mesylate (5) MsCl (0.43 ml, 5.6 mmol) was added dropwise to a stirred solution of **4** (760 mg, 0.93 mmol), DMAP (340 mg, 2.79 mmol) and Et_3N (0.38 ml, 2.8 mmol) in CH_2Cl_2 (50 ml) at −78 °C. The mixture was stirred at −78 °C for 1 h, diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1) to give **5** (797 mg, 96%) as an amorphous powder. ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.10 (3H, d, J = 7.5 Hz, Ala^4-H$_\beta$), 1.30 (3H, d, J = 6.9 Hz, Ala^1-H$_\beta$), 1.50—1.90 (4H, m, Nva^2-H$_\beta$ and Nva^2-H$_\gamma$), 2.63 (1H, dd, J = 11.2, 3.1 Hz, Ala^1-H$_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.92 (3H, s, Tyr^3-NMe), 2.92—3.09 (2H, m, Tyr^6-H$_\beta$), 3.02 (3H, s, Nva^2-OMs), 3.13 (3H, s, Tyr^5-NMe), 3.31 (1H, dd, J = 14.6, 11.1 Hz, Tyr^3-H$_{\beta a}$), 3.41 (1H, dd, J = 14.6, 4.6 Hz, Tyr^3-H$_{\beta b}$), 3.63 (1H, dd, J = 11.1, 4.6 Hz, Tyr^3-H$_\alpha$), 3.66 (1H, t, J = 11.2 Hz, Tyr^5-H$_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.25 (2H, t, J = 5.9 Hz, Nva^2-H$_\delta$), 4.34 (1H, d, J = 2.1 Hz, Tyr^6-H$_{\delta a}$), 4.39 (1H, dq, J = 8.9, 6.9 Hz, Ala^1-H$_\alpha$), 4.53 (1H, dd, J = 12.0, 4.0 Hz, Tyr^6-H$_\alpha$), 4.72 (1H, qd, J = 7.5, 7.2 Hz, Ala^4-H$_\alpha$), 4.79—4.84 (1H, m, Nva^2-H$_\alpha$), 5.38 (1H, dd, J = 11.2, 3.1 Hz, Tyr^5-H$_\alpha$), 6.37 (1H, d, J = 6.9 Hz, Nva^2-NH), 6.57 (1H, dd, J = 8.4, 2.1 Hz, Tyr^6-H$_{\delta b}$), 6.60 (1H, d, J = 8.9 Hz, Ala^1-NH), 6.70 (1H, d, J = 7.2 Hz, Ala^4-NH), 6.80 (1H, d, J = 8.4 Hz, Tyr^6-H$_\varepsilon$), 6.84 (2H, d, J = 8.6 Hz, Tyr^3-H$_\varepsilon$), 6.87 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.05 (2H, d, J = 8.6 Hz, Tyr^3-H$_\delta$), 7.20 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.26 (1H, dd, J = 8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.41 (1H, dd, J = 8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

[2-Proline]-RA-VII (6) A mixture of **5** (35.8 mg, 0.040 mmol), n-Bu_4NBr (6.5 mg, 0.020 mmol), CH_2Cl_2 (4 ml) and 50% NaOH (1 ml) was stirred vigorously at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **6** (26.1 mg, 82%) as a colorless powder, mp 294—295 °C, $[\alpha]_D$ −148.1° (c = 0.11, $CHCl_3$). IR ν ($CHCl_3$): 3400, 1680, 1651 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 225 (4.52), 277 (3.63), 283 (3.56). High-resolution FAB-MS Calcd for $C_{43}H_{53}N_6O_9$: 797.3910 $[M+H]^+$. Found: 797.3873. FAB-Ms m/z (%): 797 (20, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, δ): 0.95 (3H, d, J = 6.7 Hz, Ala^4-H$_\beta$), 1.26 (3H, d, J = 6.7 Hz, Ala^1-H$_\beta$), 1.95—2.05 (3H, m, Pro^2-H$_{\beta a}$ and Pro^2-H$_\gamma$), 2.59—2.63 (2H, m, Pro^2-H$_{\beta b}$ and Tyr^5-H$_{\beta a}$ (pro-*R*)), 2.78 (3H, s, Tyr^6-NMe), 2.85 (1H, dd, J = 17.9, 3.7 Hz, Tyr^6-H$_{\beta a}$ (pro-*S*)), 3.06 (3H, s, Tyr^5-NMe), 3.17 (1H, dd, J = 17.9, 12.0 Hz, Tyr^6-H$_{\beta b}$ (pro-*R*)), 3.25 (1H, dd, J = 14.1, 10.9 Hz, Tyr^3-H$_{\beta a}$), 3.38 (1H, dd, J = 14.1, 4.5 Hz, Tyr^3-H$_{\beta b}$), 3.50 (1H, dt, J = 9.7, 7.3 Hz, Pro^2-H$_{\delta a}$), 3.58 (1H, dd, J = 10.9, 4.5 Hz, Tyr^3-H$_\alpha$), 3.63 (1H, dd, J = 9.7, 4.4 Hz, Pro^2-H$_{\delta b}$), 3.71 (1H, t, J = 11.1 Hz, Tyr^5-H$_{\beta b}$ (pro-*S*)), 3.78 (3H, s, Tyr^3-OMe), 3.92 (3H, s, Tyr^6-OMe), 4.32 (1H, d, J = 1.8 Hz, Tyr^6-H$_{\delta a}$), 4.50 (1H, dq, J = 13.5, 6.7 Hz, Ala^1-H$_\alpha$), 4.55 (1H, dd, J = 12.0, 3.7 Hz, Tyr^6-H$_\alpha$), 4.72 (1H, dd, J = 7.8, 2.9 Hz, Pro^2-H$_\alpha$), 4.89 (1H, td, J = 15.5, 6.7 Hz, Ala^4-H$_\alpha$), 5.49 (1H, dd, J = 11.1, 2.5 Hz, Tyr^5-H$_\alpha$), 6.56 (1H, dd, J = 8.3, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.79 (1H, d, J = 8.3 Hz, Tyr^6-H$_\varepsilon$), 6.82—6.86 (2H, m, Ala^1-NH, Ala^4-NH), 6.83 (2H, d, J = 8.5 Hz, Tyr^3-H$_\varepsilon$), 6.85 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.05 (2H, d, J = 8.5 Hz, Tyr^3-H$_\delta$), 7.19 (1H, dd, J = 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J = 8.4, 2.1 Hz, Tyr^5-H$_{\delta b}$), 7.40 (1H, dd, J = 8.4, 2.1 Hz, Tyr^5-H$_{\delta b}$).

[2-Allylglycine]-RA-VII (7) A mixture of **5** (89.3 mg, 0.10 mmol), o-$NO_2PhSeCN$ (90.8 mg, 0.40 mmol), $NaBH_4$ (18.2 mg, 0.48 mmol) and EtOH (10 ml) was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1) to give the selenide. 35% H_2O_2 (2 ml) was added to the selenide dissolved in THF–EtOH (1 : 1, 20 ml), and the mixture was stirred

at room temperature for 12 h. Dimethyl sulfide was added to the mixture at room temperature to decompose excess H_2O_2. The whole was stirred for 12 h, and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1), followed by recrystallization from MeOH–isopropyl ether to give **7** (75.1 mg, 94%) as a colorless powder, mp 227—228 °C, $[\alpha]_D$ −199.4° (c=0.10, $CHCl_3$). IR ν ($CHCl_3$): 3413, 1674, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 220 (4.60), 227 (3.78), 283 (3.71). High-resolution FAB-MS Calcd for $C_{43}H_{53}N_6O_9$: 797.3874 $[M+H]^+$, Found: 797.3918. MS m/z (%): 796 (20, M^+), 121 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.13 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.29 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 2.42—2.54 (2H, m, Aa^2-H$_\beta$), 2.63 (1H, dd, J=11.3, 2.9 Hz, Tyr^5-H$_{\beta a}$), 2.68 (3H, s, Tyr^6-NMe), 2.92 (3H, s, Tyr^3-NMe), 3.13 (3H, s, Tyr^5-NMe), 3.29 (1H, dd, J=14.2, 11.1 Hz, Tyr^3-H$_{\beta a}$), 3.45 (1H, dd, J=14.2, 4.2 Hz, Tyr^3-H$_{\beta b}$), 3.60 (1H, dd, J=11.1, 4.2 Hz, Tyr^3-H$_\alpha$), 3.67 (1H, t, J=11.3 Hz, Tyr^5-H$_{\beta b}$), 3.79 (3H, s, Tyr^3-OMe), 3.94 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=2.1 Hz, Tyr^6-H$_{\delta a}$), 4.37 (1H, t, J=6.9 Hz, Ala^1-H$_\alpha$), 4.54 (1H, dd, J=12.0, 3.9 Hz, Tyr^6-H$_\alpha$), 4.74 (1H, dq, J=7.5, 6.7 Hz, Ala^4-H$_\alpha$), 4.84 (1H, m, Aa^2-H$_\alpha$), 5.18 (1H, dd, J=10.3, 1.4 Hz, Aa^2-H$_{\delta a}$), 5.22 (1H, dd, J=17.1, 1.4 Hz, Aa^2-H$_{\delta b}$), 5.40 (1H, dd, J=11.4, 3.2 Hz, Tyr^5-H$_\alpha$), 5.67—5.77 (1H, m, Aa^2-H$_\gamma$), 6.16 (1H, d, J=8.7 Hz, Aa^2-NH), 6.38 (1H, d, J=6.9 Hz, Ala^1-NH), 6.58 (1H, dd, J=8.3, 2.1 Hz, Tyr^6-H$_{\delta b}$), 6.69 (1H, d, J=7.5 Hz, Ala^4-NH), 6.80 (1H, d, J=8.3 Hz, Tyr^6-H$_\varepsilon$), 6.83 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.87 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.08 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.26 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.41 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\delta b}$).

[2-(β-Formylalanine)]-RA-VII (8) OsO_4 (20.0 mg, 0.080 mmol) and $NaIO_4$ (160.4 mg, 0.75 mmol) were added to a solution of **7** (119.5 mg, 0.15 mmol) in 1,4-dioxane–H_2O (4:1, 10 ml) and the mixture was stirred at room temperature for 18 h. The white precipitate was removed by filtration, then the filtrate was diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1), followed by recrystallization from MeOH–isopropyl ether to give **8** (91.8 mg, 76%) as a colorless powder, mp 322—323 °C, $[\alpha]_D$ −129.8° (c=0.13, $CHCl_3$). IR ν ($CHCl_3$): 3405, 1725, 1673, 1634 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 224 (4.44), 277 (3.64), 283 (3.58). High-resolution FAB-MS Calcd for $C_{42}H_{51}N_6O_{10}$: 799.3667 $[M+H]^+$. Found: 799.3722. MS m/z (%): 798 (100, M^+). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.12 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.28 (3H, d, J=7.0 Hz, Ala^1-H$_\beta$), 2.63 (1H, dd, J=11.4, 3.2 Hz, Tyr^5-H$_{\beta a}$), 2.66 (3H, s, Tyr^6-NMe), 2.83 (1H, dd, J=18.5, 5.0 Hz, Aa^2-H$_{\beta a}$), 2.92—2.95 (1H, m, Tyr^6-H$_{\beta a}$), 3.04—3.10 (1H, m, Tyr^6-H$_{\beta b}$), 3.12 (3H, s, Tyr^3-NMe), 3.15 (1H, dd, J=14.2, 10.3 Hz, Tyr^3-H$_{\beta a}$), 3.17 (3H, s, Tyr^5-NMe), 3.23 (1H, dd, J=18.5, 9.2 Hz, Aa^2-H$_{\beta b}$), 3.42 (1H, dd, J=14.2, 3.9 Hz, Tyr^3-H$_{\beta b}$), 3.58 (1H, dd, J=10.3, 3.9 Hz, Tyr^3-H$_\alpha$), 3.66 (1H, t, J=11.4 Hz, Tyr^5-H$_{\beta b}$). 3.79 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.8 Hz, Tyr^6-H$_{\delta a}$), 4.36 (1H, dq, J=7.1, 7.0 Hz, Ala^1-H$_\alpha$), 4.56 (1H, dd, J=11.9, 3.8 Hz, Tyr^6-H$_\alpha$), 4.70 (1H, dq, J=7.3, 6.7 Hz, Ala^4-H$_\alpha$), 5.24 (1H, ddd, J=9.2, 8.6, 5.9 Hz, Aa^2-H$_\alpha$), 5.38 (1H, dd, J=11.4, 3.2 Hz, Tyr^3-H$_\alpha$), 6.40 (1H, d, J=7.1 Hz, Ala^1-NH), 6.58 (1H, dd, J=8.4, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.77 (1H, d, J=7.3 Hz, Ala^4-NH), 6.80 (1H, d, J=8.4 Hz, Tyr^6-H$_\varepsilon$), 6.87 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.87 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$). 6.94 (1H, d, J=8.6 Hz, Ala^2-NH), 7.05 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.40 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$), 9.74 (1H, s, Aa^2-CHO).

[2-[(*E*)-2-Amino-4-hexene-1,6-dicarboxylic acid]]-RA-VII Ethyl Ester (9) Ethyl diethylphosphonoacetate (126 μl, 0.63 mmol), NaH (60%, 25.2 mg, 0.63 mmol) and 18-crown-6 (40.4 mg, 0.15 mmol) were added to a solution of **8** (235.8 mg, 0.29 mmol) in THF (15 ml) at 0 °C. The mixture was stirred at room temperature for 12 h, then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1) to give **9** (185.7 mg, 72%) as an amorphous powder. ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.12 (3H, d, J=6.6 Hz, Ala^4-H$_\beta$), 1.27 (3H, t, J=7.1 Hz, Aa^2-COOCH$_2$CH$_3$), 1.28 (3H, d, J=7.0 Hz, Ala^1-H$_\beta$), 2.61—2.64 (3H, m, Aa^2-H$_\beta$ and Tyr^5-H$_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.91 (3H, s, Tyr^3-NMe), 2.95 (1H, dd, J=20.3, 4.1 Hz, Tyr^6-H$_{\beta a}$), 3.08 (1H, dd, J=20.3, 12.1 Hz, Tyr^6-H$_{\beta b}$), 3.13 (3H, s, Tyr^5-NMe), 3.26 (1H, dd, J=14.1, 11.2 Hz, Tyr^3-H$_{\beta a}$), 3.42 (1H, dd, J=14.1, 4.1 Hz, Tyr^3-H$_{\beta b}$), 3.58 (1H, dd, J=11.2, 4.1 Hz, Tyr^3-H$_\alpha$), 3.66 (1H, t, J=11.4 Hz, Tyr^5-H$_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.20 (2H, q, J=7.1 Hz, Aa^2-COOCH$_2$CH$_3$), 4.33 (1H, d, J=1.9 Hz, Tyr^6-H$_{\delta a}$), 4.39 (1H, m, Ala^1-H$_\alpha$), 4.54 (1H, dd, J=12.1, 4.1 Hz, Tyr^6-H$_\alpha$), 4.72 (1H, dq, J=7.4, 6.6 Hz, Ala^4-H$_\alpha$), 4.92 (1H, m, Aa^2-H$_\alpha$), 5.38 (1H, dd, J=11.4, 3.1 Hz, Tyr^5-H$_\alpha$), 5.97 (1H, d, J=15.6 Hz, Aa^2-H$_\delta$), 6.34 (1H, d, J=7.0 Hz, Ala^1-NH), 6.50 (1H, d, J=9.5 Hz, Aa^2-NH), 6.57 (1H, dd, J=8.4, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.69 (1H, d, J=7.4 Hz, Ala^4-NH), 6.78—6.89 (1H, m, Aa^2-H$_\gamma$), 6.79 (1H, dd, J=8.4 Hz, Tyr^6-H$_\varepsilon$), 6.83 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.87 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.03 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.20 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.26 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.42 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

[2-(ε-Hydroxynorleucine)]-RA-VII (10) Palladium (10%) on activated carbon (100 mg) was added to a solution of **9** (185.7 mg, 0.21 mmol) in 1,4-dioxane (20 ml), and the mixture was stirred vigorously at room temperature for 24 h under hydrogen. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in THF (30 ml), and $LiBH_4$ (2 M in THF, 1.05 ml, 2.3 mmol) was added to the solution at 0 °C. Stirring was continued at room temperature for 24 h, then the mixture was concentrated *in vacuo*. The residue was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1), followed by recrystallization from MeOH–isopropyl ether to give **10** (75.4 mg, 31%) as a colorless powder, mp 227—228 °C, $[\alpha]_D$ −176.3° (c=0.14, $CHCl_3$). IR ν ($CHCl_3$): 3403, 1674, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 221 (4.49), 276 (3.62), 282 (3.56). High-resolution FAB-MS Calcd for $C_{44}H_{57}N_6O_{10}$: 829.4136 $[M+H]^+$. Found: 829.4054. FAB-MS m/z (%): 829 (10, $[M+H]^+$). 121 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.13 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.31 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 1.33—1.46 (2H, m, Nle^2-H$_\beta$), 1.54—1.78 (4H, m, Nle^2-H$_\gamma$ and Nle^2-H$_\delta$), 2.63 (1H, dd, J=11.4, 3.2 Hz, Tyr^5-H$_\beta$), 2.68 (3H, s, Tyr^6-NMe), 2.93 (3H, s, Tyr^3-NMe), 2.95 (1H, dd, J=18.1, 3.9 Hz, Tyr^6-H$_{\beta a}$), 3.10 (1H, dd, J=18.1, 12.0 Hz, Tyr^6-H$_{\beta b}$), 3.13 (3H, s, Tyr^5-NMe), 3.31 (1H, dd, J=14.2, 11.1 Hz, Tyr^3-H$_{\beta a}$), 3.44 (1H, dd, J=14.2, 4.3 Hz, Tyr^3-H$_{\beta b}$), 3.62 (1H, dd, J=11.1, 4.3 Hz, Tyr^3-H$_\alpha$), 3.64—3.70 (3H, m, Nle^2-H$_\varepsilon$ and Tyr^5-H$_{\beta b}$), 3.79 (3H, s, Tyr^3-OMe), 3.94 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.9 Hz, Tyr^6-H$_{\delta a}$), 4.37 (1H, dq, J=6.9, 6.8 Hz, Ala^1-H$_\alpha$), 4.54 (1H, dd, J=12.0, 3.9 Hz, Tyr^6-H$_\alpha$), 4.70—4.79 (2H, m, Nle^2-H$_\alpha$ and Ala^4-H$_\alpha$), 5.41 (1H, dd, J=11.4, 3.2 Hz, Tyr^5-H$_\alpha$), 6.42 (1H, d, J=6.8 Hz, Ala^1-NH), 6.45 (1H, d, J=8.6 Hz, Nle^2-NH), 6.58 (1H, dd, J=8.4, 1.9 Hz, Tyr^6-H$_{\delta b}$), 6.72 (1H, d, J=7.5 Hz, Ala^4-NH), 6.80 (1H, d, J=8.4 Hz, Tyr^6-H$_\varepsilon$), 6.84 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.88 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.08 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.26 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.42 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

[2-Pipecolic acid]-RA-VII (11) MsCl (42 μl, 0.54 mmol) was added dropwise to a solution of **10** (75.3 mg, 0.090 mmol), DMAP (33.0 mg, 0.27 mmol) and Et_3N (38 μl, 0.27 mmol) in CH_2Cl_2 (8 ml) at −78 °C, and stirring was continued for 1 h. The mixture was diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1) to afford the mesylate (58.1 mg) as an amorphous powder. A mixture of the mesylate (58.1 mg, 0.064 mmol), n-Bu_4NBr (10.3 mg, 0.032 mmol), CH_2Cl_2 (4 ml) and 50% NaOH (1 ml) was stirred vigorously at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12:2:1), followed by recrystallization from MeOH–isopropyl ether to give **11** (41.6 mg, 56% from **10**) as a colorless powder, mp 250—251 °C, $[\alpha]_D$ −210.2° (c=0.11, $CHCl_3$). IR ν ($CHCl_3$): 3392, 1681, 1646 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 220 (4.52), 277 (3.59), 282 (3.54). High-resolution FAB-MS Calcd for $C_{44}H_{55}N_6O_9$: 811.4031 $[M+H]^+$. Found: 811.4089. FAB-MS m/z (%): 811 (5, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, δ): 0.97 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.24 (3H, d, J=6.9 Hz, Ala-^{1}H$_\beta$), 1.45—1.52 (1H, m, Pip^2-H$_{\delta a}$), 1.63—1.72 (2H, m, Pip^2-H$_\beta$), 1.77—1.81 (1H, m, Pip^2-H$_{\gamma a}$), 1.85—1.88 (1H, m, Pip^2-H$_{\delta b}$), 2.05—2.10 (1H, m, Pip^2-H$_{\gamma b}$), 2.60 (1H, dd, J=11.2, 2.7 Hz, Tyr^5-H$_{\beta a}$), 2.74 (3H, s, Tyr^6-NMe), 2.83 (3H, s, Tyr^3-NMe), 2.89 (1H, dd, J=18.0, 3.7 Hz, Tyr^6-H$_{\beta a}$), 3.06 (3H, s, Tyr^5-NMe), 3.13 (1H, dd, J=18.0, 12.0 Hz, Tyr^6-H$_{\beta b}$), 3.32 (1H, dd, J=14.4, 10.3 Hz, Tyr^3-H$_{\beta a}$), 3.35 (1H, dd, J=14.4, 5.3 Hz, Tyr^3-H$_{\beta b}$), 3.53 (1H, m, Pip^2-H$_{\varepsilon a}$), 3.53 (1H, dd, J=10.3, 5.3 Hz, Tyr^3-H$_\alpha$), 3.68 (1H, t, J=11.2 Hz, Tyr^5-H$_{\beta b}$), 3.92 (1H,

dd, J=22.6, 2.3 Hz, Pip^2-H$_{\varepsilon b}$), 4.33 (1H, d, J=1.8 Hz, Tyr^6-H$_{\delta a}$), 4.54 (1H, dd, J=12.0, 3.7 Hz, Tyr^6-H$_\alpha$), 4.64 (1H, qd, J=6.9, 6.7 Hz, Ala^1-H$_\alpha$), 4.79 (1H, dq, J=8.2, 6.7 Hz, Ala^4-H$_\alpha$), 5.08 (1H, dd, J=6.3, 1.8 Hz, Pip^2-H$_\alpha$), 5.45 (1H, dd, J=11.2, 2.7 Hz, Tyr^5-H$_\alpha$), 6.55 (1H, dd, J=8.6, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.67 (1H, d, J=8.2 Hz, Ala^4-NH), 6.73 (1H, d, J=6.7 Hz, Ala^1-NH), 6.82 (1H, d, J=8.6 Hz, Tyr^6-H$_\varepsilon$), 6.82 (2H, d, J=8.5 Hz, Tyr^3-H$_\varepsilon$), 6.86 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.04 (2H, d, J=8.5 Hz, Tyr^3-H$_\delta$), 7.19 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-H$_{\delta a}$), 7.40 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-H$_{\delta b}$).

[2-Norvaline]-RA-VII (12) Palladium (10%) on activated carbon (20 mg) was added to a solution of **7** (39.8 mg, 0.050 mmol) in 1,4-dioxane (5 ml), and the mixture was stirred vigorously at room temperature for 24 h under hydrogen. The mixture was filtered, and the filtrate was concentrated *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH to give **12** (34.8 mg, 87%) as a colorless powder, mp 227—228 °C, $[\alpha]_D$ −204.5° (c=0.16, $CHCl_3$). IR ν ($CHCl_3$): 3410, 1674, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 223 (4.73), 277 (3.85), 282 (3.80). High-resolution FAB-MS Calcd for $C_{43}H_{55}N_6O_9$: 799.4031 $[M+H]^+$. Found: 799.4043. FAB-MS m/z (%): 799 (20, $[M+H]^+$). 164 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 0.93 (3H, t, J=7.3 Hz, Nva^2-H$_\beta$), 1.11 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.28 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 1.27—1.36 (2H, m, Nva^2-H$_\beta$), 2.63 (1H, dd, J=11.7, 3.1 Hz, Tyr^5-H$_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.91 (3H, s, Tyr^3-NMe), 2.96—3.08 (2H, m, Tyr^6-H$_\beta$), 3.11 (3H, s, Tyr^5-NMe), 3.29 (1H, dd, J=14.2, 11.0 Hz, Tyr^3-H$_{\beta a}$), 3.43 (1H, dd, J=14.2, 4.3 Hz, Tyr^5-H$_{\beta b}$), 3.61 (1H, dd, J=11.0, 4.3 Hz, Tyr^3-H$_\alpha$), 3.65 (1H, t, J=11.7 Hz, Tyr^5-H$_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.92 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=2.0 Hz, Tyr^6-H$_{\delta a}$), 4.37 (1H, t, J=6.9 Hz, Ala^1-H$_\alpha$), 4.53 (1H, dd, J=12.0, 3.9 Hz, Tyr^6-H$_\alpha$), 4.71—4.79 (2H, m, Nva^2-H$_\alpha$ and Ala^4-H$_\alpha$), 5.39 (1H, dd, J=11.7, 3.1 Hz, Tyr^5-H$_\alpha$), 6.42 (1H, d, J=6.9 Hz, Ala^1-NH), 6.56 (1H, d, J=8.4 Hz, Nva^2-NH), 6.57 (1H, dd, J=8.4, 2.0 Hz, Tyr^6-H$_{\delta b}$), 6.69 (1H, d, J=7.5 Hz, Ala^4-NH), 6.79 (1H, d, J=8.4 Hz, Tyr^6-H$_\varepsilon$), 6.82 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.86 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.07 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.20 (1H, dd, 8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J=8.4, 2.3 Hz, Tyr^6-H$_{\delta a}$), 7.41 (1H, dd, J=8.4, 2.3 Hz, Tyr^6-H$_{\delta b}$).

[2-(δ-Azidonorvaline)]-RA-VII (13) NaN_3 (588.7 mg, 9.0 mmol), 18-crown-6 (158.6 mg, 0.60 mmol) and **5** (268.9 mg, 0.30 mmol) were dissolved in CH_2Cl_2 (10 ml) and the solution was refluxed for 9 h. The mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **13** (218.5 mg, 86%) as a colorless powder, mp 211—212 °C, $[\alpha]_D$ −209.5°C (c=0.11, $CHCl_3$). IR ν ($CHCl_3$): 3420, 2103, 1677, 1634 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 225 (4.53), 277 (3.66), 285 (3.60). High-resolution FAB-MS Calcd for $C_{43}H_{53}N_6O_9$: 840.4043 $[M+H]^+$. Found: 840.4043. FAB-MS m/z(%): 840 (70, $[M+H]^+$), 164 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.14 (3H, d, J=6.6 Hz, Ala^4-H$_\beta$), 1.31 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 1.54—1.63 (2H, m, Nva^2-H$_\beta$), 1.76—1.82 (2H, m, Nva^2-H$_\gamma$), 2.64 (1H, dd, J=11.2, 3.0 Hz, Tyr^5-H$_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.91 (3H, s, Tyr^3-NMe), 2.96 (1H, dd, J=17.7, 3.7 Hz, Tyr^6-H$_{\beta a}$), 3.10 (1H, dd, J=17.7, 11.8 Hz, Tyr^6-H$_{\beta b}$), 3.14 (3H, s, Tyr^5-NMe), 3.33 (1H, dd, J=14.2, 11.1 Hz, Tyr^3-H$_{\beta a}$), 3.33—3.37 (2H, m, Nva^2-H$_\delta$), 3.44 (1H, dd, J=14.2, 4.4 Hz, Tyr^3-H$_{\beta b}$), 3.63 (1H, dd, J=11.1, 4.4 Hz, Tyr^3-H$_\alpha$), 3.67 (1H, t, J=11.2 Hz, Tyr^5-H$_{\beta b}$), 3.79 (3H, s, Tyr^3-OMe), 3.94 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.8 Hz, Tyr^6-H$_{\delta a}$), 4.37 (1H, dq, J=8.8, 6.6 Hz, Ala^1-H$_\alpha$), 4.53 (1H, dd, J=11.8, 3.7 Hz, Tyr^6-H$_\alpha$), 4.70 (1H, dq, J=7.3, 6.9 Hz, Ala^4-H$_\alpha$), 4.81 (1H, m, Nva^2-H$_\alpha$), 5.39 (1H, dd, J=11.2, 3.0 Hz, Tyr^5-H$_\alpha$), 6.24 (1H, d, J=8.8 Hz, Ala^1-NH), 6.35 (1H, d, J=6.8 Hz, Ala^2-NH), 6.58 (1H, dd, J=8.4, 1.8 Hz, Tyr^6-H$_{\delta b}$), 6.69 (1H, d, J=7.3 Hz, Ala^4-NH), 6.80 (1H, d, J=8.4 Hz, Tyr^6-H$_\varepsilon$), 6.84 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.88 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.07 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.27 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.42 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

[2-Ornithine]-RA-VII (14) A mixture of **13** (153.5 mg, 0.18 mmol), Ph_3P (94.4 mg, 0.36 mmol), H_2O (50 μl) and THF (10 ml) was stirred at room temperature for 62 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **14** (43.8 mg, 29%) as a colorless powder, mp 195—197 °C, $[\alpha]_D$ −129.8° (c=0.13, $CHCl_3$). IR ν ($CHCl_3$): 3392, 1662 cm^{-1}. UN λ_{max} (EtOH) nm (log ε): 220 (4.35), 277 (3.45), 282 (3.39). High-resolution FAB-MS Calcd for $C_{43}H_{56}N_7O_9$: 814.4140 $[M+H]^+$. Found: 814.4089. FAB-MS m/z (%): 814 (20, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.06 (3H, d, J=6.6 Hz, Ala^4-H$_\beta$), 1.35 (3H, d, J=6.6 Hz, Ala^1-H$_\beta$), 1.77—1.79 (2H, m, Orn^2-H$_\gamma$), 1.97—2.06 (2H, m, Orn^2-H$_\delta$), 2.19—2.27 (2H, m, Orn^2-H$_\beta$), 2.61 (1H, dd, J=11.2, 2.5 Hz, Tyr^5-H$_{\beta a}$), 2.68 (3H, s, Tyr^6-NMe), 2.89—2.94 (1H, m, Tyr^6-H$_{\beta a}$), 2.96 (3H, s, Tyr^3-NMe), 3.06—3.10 (1H, m, Tyr^6-H$_{\beta b}$), 3.08 (3H, s, Tyr^3-NMe), 3.20 (1H, dd, J=13.5, 10.8 Hz, Tyr^3-H$_{\beta a}$), 3.34 (1H, dd, J=13.5, 4.4 Hz, Tyr^3-H$_{\beta b}$), 3.61 (1H, dd, J=10.8, 4.4 Hz, Tyr^3-H$_\alpha$), 3.71 (4H, m, Tyr^3-OMe and Tyr^5-H$_{\beta b}$), 3.93 (3H, s, Tyr^6-OMe), 4.35 (1H, d, J=1.4 Hz, Tyr^6-H$_{\delta a}$), 4.49—4.56 (2H, m, Ala^1-H$_\alpha$ and Tyr^6-H$_\alpha$), 4.61 (1H, m, Orn^2-H$_\alpha$), 4.73 (1H, dq, J=8.5, 6.6 Hz, Ala^4-H$_\alpha$), 5.42 (1H, dd, J=11.2, 2.5 Hz, Tyr^5-H$_\alpha$), 6.43 (1H, d, J=6.9 Hz, Ala^1-NH), 6.57 (1H, dd, J=8.2, 1.4 Hz, Tyr^6-H$_{\delta b}$), 6.79 (1H, d, J=8.2 Hz, Tyr^6-H$_\varepsilon$), 6.81 (2H, d, J=8.3 Hz, Tyr^3-H$_\varepsilon$), 6.86 (1H, d, J=8.5 Hz, Ala^4-NH), 6.87 (1H, dd, J=8.4, 2.3 Hz, Tyr^5-H$_{\varepsilon a}$), 7.09 (2H, d, J=8.3 Hz, Tyr^3-H$_\delta$), 7.20 (1H, dd, J=8.5, 2.3 Hz, Tyr^5-H$_{\varepsilon b}$), 7.25 (1H, dd, J=8.4, 2.0 Hz, Tyr^5-H$_{\delta a}$), 7.40 (1H, dd, J=8.5, 2.0 Hz, Tyr^5-H$_{\delta b}$), 8.76 (1H, d, J=6.2 Hz, Orn^2-NH).

[2-Aspartic Acid]-RA-VII (15). a) Oxidation of 8 with AgO AgO (24.8 mg, 0.20 mmol) was added to a solution of **8** (39.9 mg, 0.50 mmol) in THF–H_2O (9 : 1, 2 ml) and the mixture was refluxed for 9 h. The mixture filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **15** (5.7 mg, 14%) as a colorless powder, mp 245—246 °C, $[\alpha]_D$ −187.0° (c=0.11, $CHCl_3$). IR ν ($CHCl_3$): 3395, 1673, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 220 (4.48), 277 (3.58), 282 (3.52). High-resolution FAB-MS Calcd for $C_{42}H_{55}N_6O_{11}$: 815.3616 $[M+H]^+$. Found: 815.3644. FAB-MS m/z (%): 815 (50, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.08 (3H, d, J=6.6 Hz, Ala^4-H$_\beta$), 1.28 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 2.62 (1H, dd, J=11.3, 2.8 Hz, Tyr^5-H$_{\beta a}$), 2.69—2.90 (4H, m, Asp^2-H$_\beta$ and Tyr^6-H$_\beta$), 2.65 (3H, s, Tyr^6-NMe), 3.05 (3H, s, Tyr^3-NMe), 3.09 (3H, s, Tyr^5-NMe), 3.16 (1H, dd, J=14.0, 10.4 Hz, Tyr^3-H$_{\beta a}$), 3.35 (1H, dd, J=14.0, 4.2 Hz, Tyr^3-H$_{\beta b}$), 3.59 (1H, dd, J=10.4, 4.2 Hz, Tyr^3-H$_\alpha$), 3.65 (1H, t, J=11.3 Hz, Tyr^5-H$_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.92 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.5 Hz, Tyr^6-H$_{\delta a}$), 4.47 (1H, dq, J=7.4, 6.9 Hz, Ala^1-H$_\alpha$), 4.57 (1H, dd, J=10.8, 4.5 Hz, Tyr^6-H$_\alpha$), 4.69 (1H, dq, J=8.5, 6.6 Hz, Ala^4-H$_\alpha$), 5.10 (1H, dd, J=13.7, 7.6 Hz, Asp^2-H$_\alpha$), 5.38 (1H, dd, J=11.3, 2.8 Hz, Tyr^5-H$_\alpha$), 6.53 (1H, d, J=7.4 Hz, Ala^1-NH), 6.56 (1H, d, J=8.5 Hz, Ala^4-NH), 6.56 (1H, dd, J=8.5, 1.5 Hz, Tyr^6-H$_{\delta b}$), 6.79 (1H, d, J=8.5 Hz, Tyr^6-H$_\varepsilon$), 6.84 (2H, d, J=8.4 Hz, Tyr^3-H$_\varepsilon$), 6.86 (1H, dd, J=8.6, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.07 (2H, d, J=8.4 Hz, Tyr^3-H$_\delta$), 7.20 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.24 (1H, dd, J=8.6, 2.1 Hz, Tyr^5-H$_{\delta a}$), 7.40 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-H$_{\delta b}$), 7.80 (1H, d, J=7.6 Hz, Asp^2-NH).

b) Oxidation of 8 with NIS NIS (42.2 mg, 0.19 mmol) and K_2CO_3 (25.9 mg, 0.19 mmol) were added to a solution of **8** (59.8 mg, 0.075 mmol) in CH_3OH (2 ml) and the mixture was stirred at room temperature for 7 h. Then 5% $Na_2S_2O_3$ was added to the mixture, and the whole was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1) to give [2-aspartic acid]-RA-VII methyl ester (**16**) (40.6 mg, 65%) as an amorphous powder. ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.12 (3H, d, J=6.7 Hz, Ala^4-H$_\beta$), 1.29 (3H, d, J=6.9 Hz, Ala^1-H$_\beta$), 2.63 (1H, dd, J=11.3, 3.0 Hz, Tyr^5-H$_{\beta a}$), 2.67 (1H, dd, J=16.6, 6.0 Hz, Asp^2-H$_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.92 (1H, dd, J=16.5, 8.4 Hz, Asp^2-H$_{\beta b}$), 2.98—3.08 (2H, m, Tyr^6-H$_\beta$), 3.10 (3H, s, Tyr^3-NMe), 3.12 (3H, s, Tyr^5-NMe), 3.24 (1H, dd, J=14.2, 10.5 Hz, Tyr^3-H$_{\beta a}$), 3.44 (1H, dd, J=14.2, 4.2 Hz, Tyr^3-H$_{\beta b}$), 3.59 (1H, dd, J=10.5, 4.2 Hz, Tyr^3-H$_\alpha$), 3.67 (1H, t, J=11.3 Hz, Tyr^5-H$_{\beta b}$), 3.73 (3H, s, Asp^2-OMe), 3.79 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.7 Hz, Tyr^6-H$_{\delta a}$), 4.38 (1H, t, J=6.9 Hz, Ala^1-H$_\alpha$), 4.56 (1H, dd, J=12.4, 3.8 Hz, Tyr^6-H$_\alpha$), 4.71 (1H, dq, J=8.7, 6.7 Hz, Ala^4-H$_\alpha$), 5.19 (1H, td, J=8.4, 6.0 Hz, Asp^2-H$_\alpha$), 5.39 (1H, dd, J=11.3, 3.0 Hz, Tyr^5-H$_\alpha$), 6.38 (1H, d, J=6.9 Hz, Ala^1-NH), 6.58 (1H, dd, J=8.4, 1.7 Hz, Tyr^6-H$_{\delta b}$), 6.70 (1H, d, J=8.7 Hz, Ala^4-NH), 6.80 (2H, d, J=8.4 Hz, Tyr^6-H$_\varepsilon$, Asp^2-NH), 6.83 (2H, d, J=8.6 Hz, Tyr^3-H$_\varepsilon$), 6.88 (1H, dd, J=8.6, 2.4 Hz, Tyr^5-H$_{\varepsilon a}$), 7.07 (2H, d, J=8.6 Hz, Tyr^3-H$_\delta$), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-H$_{\varepsilon b}$), 7.26 (1H, dd, J=8.6, 2.2 Hz, Tyr^5-H$_{\delta a}$), 7.41 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-H$_{\delta b}$).

$LiOH \cdot H_2O$ (3.3 mg, 0.079 mmol) was added to a solution of **16** (40.5 mg, 0.048 mmol) in MeOH–H_2O (2 : 1, 2 ml) and the mixture was stirred at room temperature for 4 h, then acidified with 1 N HCl and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **15** (36.3 mg, 91%) as a colorless powder.

[2-Homoserine]-RA-VII (17) $NaBH_4$ (51.5 mg, 1.36 mmol) was added to a solution of **8** (272.0 mg, 0.34 mmol) in 1,4-dioxane (20 ml), and the mixture was stirred at room temperature for 3 h, then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **17** (246.6 mg, 90%) as a colorless powder, mp 261—262 °C, $[\alpha]_D$ −202.7° (c=0.09, $CHCl_3$). IR ν ($CHCl_3$): 3401, 1669, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 223 (4.55), 277 (3.64), 282 (3.60). High-resolution FAB-MS Calcd for $C_{42}H_{53}N_6O_{10}$: 801.3823 $[M+H]^+$. Found: 801.3763. FAB-MS m/z (%): 801 (15, $[M+H]^+$), 337 (100). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.15 (3H, d, J=6.6 Hz, Ala^4-H_β), 1.31 (3H, d, J=6.9 Hz, Ala^1-H_β), 1.93 (2H, m, Hse^2-H_β), 2.64 (1H, dd, J=11.4, 2.7 Hz, Tyr^5-$H_{\beta a}$), 2.66 (3H, s, Tyr^6-NMe), 2.93 (3H, s, Tyr^3-NMe), 3.00 (1H, dd, J=18.3, 3.7 Hz, Tyr^6-$H_{\beta a}$), 3.08 (1H, dd, J=18.3, 12.0 Hz, Tyr^6-$H_{\beta b}$), 3.14 (3H, s, Tyr^5-NMe), 3.23 (1H, dd, J=14.2, 10.9 Hz, Tyr^3-$H_{\beta a}$), 3.42 (1H, dd, J=14.2, 4.3 Hz, Tyr^3-$H_{\beta b}$), 3.59 (1H, dd, J=10.9, 4.3 Hz, Tyr^3-H_α), 3.65 (1H, t, J=11.4 Hz, Tyr^5-$H_{\beta b}$), 3.70—3.79 (2H, m, Hse^2-H_γ), 3.80 (3H, s, Tyr^3-OMe), 3.90 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=2.0 Hz, Tyr^6-$H_{\delta a}$), 4.41 (1H, dq, J=7.2, 6.9 Hz, Ala^4-H_α), 4.52 (1H, dd, J=12.0, 3.7 Hz, Tyr^6-H_α), 4.73 (1H, dq, J=7.4, 6.6 Hz, Ala^4-H_α), 5.01 (1H, td, J=7.7, 7.0 Hz, Hse^2-H_α), 5.38 (1H, dd, J=11.4, 2.7 Hz, Tyr^5-H_α), 6.50 (1H, d, J=7.2 Hz, Ala^1-NH), 6.57 (1H, dd, J=8.4, 2.0 Hz, Tyr^6-$H_{\delta b}$), 6.73 (1H, d, J=7.4 Hz, Ala^4-NH), 6.76 (1H, d, J=8.4 Hz, Tyr^6-H_ε), 6.81—6.85 (1H, m, Hse^2-NH), 6.83 (2H, d, J=8.6 Hz, Tyr^3-H_ε), 6.88 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-$H_{\varepsilon a}$), 7.03 (2H, d, J=8.6 Hz, Tyr^3-H_δ), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-$H_{\varepsilon b}$), 7.27 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\delta a}$), 7.43 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\delta b}$).

[2-Homoserine]-RA-VII Mesylate (18) MsCl (30 μl, 0.34 mmol) was added dropwise to a solution of **17** (46.4 mg, 0.058 mmol), DMAP (21.2 mg, 0.17 mmol) and Et_3N (24 μl, 0.17 mmol) in CH_2Cl_2 (5 ml) at −78 °C. The mixture was stirred at −78 °C for 2 h, then diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1) to give **18** (36.1 mg, 71%) as an amorphous powder. ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.08 (3H, d, J=6.6 Hz, Ala^4-H_β), 1.28 (3H, d, J=7.0 Hz, Ala^1-H_β), 2.08—2.14 (2H, m, Hse^2-H_β), 2.60—2.62 (1H, m, Tyr^5-$H_{\beta a}$), 2.64 (3H, s, Tyr^6-NMe), 2.93 (3H, s, Tyr^3-NMe), 2.96 (3H, s, Hse^2-OMs), 2.98—3.07 (2H, m, Tyr^6-H_β), 3.10 (3H, s, Tyr^5-NMe), 3.26 (1H, dd, J=14.1, 11.0 Hz, Tyr^3-$H_{\beta a}$), 3.38 (1H, dd, J=14.1, 4.8 Hz, Tyr^3-$H_{\beta b}$), 3.61 (1H, dd, J=11.0, 4.8 Hz, Tyr^3-H_α), 3.63 (1H, t, J=11.2 Hz, Tyr^5-$H_{\beta b}$), 3.76 (3H, s, Tyr^3-OMe), 3.90 (3H, s, Tyr^6-OMe), 4.14—4.20 (2H, m, Hse^2-H_γ), 4.31 (1H, d, J=1.9 Hz, Tyr^6-$H_{\delta a}$), 4.40 (1H, m, Ala^1-H_α), 4.51 (1H, dd, J=11.7, 3.8 Hz, Tyr^6-H_α), 4.70 (1H, dq, J=7.3, 6.6 Hz, Ala^4-H_α), 4.95 (1H, dd, J=11.3, 3.2 Hz, Hse^2-H_α), 5.36 (1H, dd, J=11.2, 3.2 Hz, Tyr^5-H_α), 6.40 (1H, d, J=7.0 Hz, Hse^2-NH), 6.54—6.57 (1H, m, Ala^1-NH), 6.55 (1H, dd, J=8.3, 1.9 Hz, Tyr^6-$H_{\delta b}$), 6.70 (1H, d, J=7.3 Hz, Ala^4-NH), 6.77 (1H, d, J=8.3 Hz, Tyr^6-H_ε), 6.82 (2H, d, J=8.5 Hz, Tyr^3-H_ε), 6.84 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\varepsilon a}$), 7.14 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\varepsilon b}$), 7.23 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\delta a}$), 7.38 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\delta b}$).

[2-Vinylglycine]-RA-VII (19) A mixture of **18** (105.0 mg, 0.12 mmol), *o*-$NO_2PhSeCN$ (54.5 mg, 0.24 mmol), $NaBH_4$ (9.1 mg, 0.24 mmol) and EtOH (10 ml) was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2. This solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1) to give the selenide. 35% H_2O_2 (2 ml) was added to the selenide dissolved in THF–EtOH (1 : 1, 20 ml), and the whole was stirred at room temperature for 12 h. Dimethyl sulfide was added to decompose excess H_2O_2, and the mixture was stirred at room temperature for 2 h. Then H_2O was added and the whole was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, and this solution was washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **19** (16.3 mg, 17%) as a colorless powder, mp 261—262 °C, $[\alpha]_D$ −202.3° (c=0.12, $CHCl_3$). IR ν ($CHCl_3$): 3400, 1675, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 219 (4.48), 277 (3.61), 282 (3.56). High-resolution FAB-MS Calcd for $C_{42}H_{51}N_6O_9$: 783.3718 $[M+H]^+$. Found: 783.3720. FAB-MS m/z (%): 783 (75, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.12 (3H, d, J=6.6 Hz, Ala^4-H_β), 1.32 (3H, d, J=6.9 Hz, Ala^1-H_β), 2.60 (1H, dd, J=11.9, 3.9 Hz, Tyr^5-$H_{\beta a}$), 2.68 (3H, s, Tyr^6-NMe), 2.87 (3H, s, Tyr^3-NMe), 2.91—3.10 (2H, m, Tyr^6-H_β), 3.12 (3H, s, Tyr^5-NMe), 3.29 (1H, dd, J=14.2, 11.0 Hz, Tyr^3-$H_{\beta a}$), 3.40 (1H, dd, J=14.2, 4.4 Hz, Tyr^3-$H_{\beta b}$), 3.57 (1H, dd, J=11.0, 4.4 Hz, Tyr^3-H_α), 3.66 (1H, t, J=11.9 Hz, Tyr^5-$H_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.8 Hz, Tyr^6-$H_{\delta a}$), 4.42 (1H, qd, J=6.9, 6.8 Hz, Ala^1-H_α), 4.54 (1H, dd, J=11.9, 3.9 Hz, Tyr^6-H_α), 4.74 (1H, dq, J=7.5, 6.6 Hz, Ala^4-H_α), 5.33—5.42 (4H, m, Aa^2-H_α, Aa^2-H_γ and Tyr^5-H_α), 5.86—5.95 (1H, m, Aa^2-H_β), 6.46 (1H, d, J=6.8 Hz, Ala^1-NH), 6.58 (1H, dd, J=8.4, 1.8 Hz, Tyr^6-$H_{\delta b}$), 6.71 (1H, d, J=7.5 Hz, Ala^4-NH), 6.76 (1H, d, J=7.5 Hz, Aa^2-NH), 6.79 (1H, d, J=8.4 Hz, Tyr^6-H_ε), 6.80 (2H, d, J=8.6 Hz, Tyr^3-H_ε), 6.87 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-$H_{\varepsilon a}$), 7.02 (2H, d, J=8.6 Hz, Tyr^3-H_δ), 7.19 (1H, dd, J=8.3, 2.4 Hz, Tyr^5-$H_{\varepsilon b}$), 7.26 (1H, dd, J=8.4, 2.1 Hz, Tyr^5-$H_{\delta a}$), 7.41 (1H, dd, J=8.3, 2.1 Hz, Tyr^5-$H_{\delta b}$).

[2-Methionine]-RA-VII (20) NaI (21.4 mg, 0.14 mmol) was added to a solution of **18** (50.0 mg, 0.057 mmol) in methyl ethyl ketone (3 ml), and the mixture was refluxed for 10 h, then concentrated *in vacuo* to leave a residue. A mixture of the residue, $NaSCH_3$ (19.9 mg, 0.28 mmol) and MeOH (4 ml) was stirred at room temperature for 0.5 h, then diluted with CH_2Cl_2, washed successively with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to leave a residue, which was chromatographed on silica gel with CH_2Cl_2–AcOEt–MeOH (12 : 2 : 1), followed by recrystallization from MeOH–isopropyl ether to give **20** (43.0 mg, 91%) as a colorless powder, mp 204—205 °C, $[\alpha]_D$ −188.2° (c=0.13, $CHCl_3$). IR ν ($CHCl_3$): 3407, 1676, 1636 cm^{-1}. UV λ_{max} (EtOH) nm (log ε): 221 (4.47), 277 (3.59), 282 (3.34). High-resolution FAB-MS Calcd for $C_{43}H_{55}N_6O_9S$: 831.3751 $[M+H]^+$. Found: 831.3754. FAB-MS m/z (%): 831 (70, $[M+H]^+$). ^{1}H-NMR ($CDCl_3$, major conformer, δ): 1.12 (3H, d, J=6.6 Hz, Ala^4-H_β), 1.30 (3H, d, J=7.0 Hz, Ala^1-H_β), 1.95—2.05 (2H, m, Met^2-H_β), 2.08 (3H, s, Met^2-SMe), 2.43—2.53 (2H, m, Met^2-H_γ), 2.64 (1H, dd, J=11.3, 3.1 Hz, Tyr^5-$H_{\beta a}$), 2.67 (3H, s, Tyr^6-NMe), 2.92—2.98 (1H, m, Tyr^6-$H_{\beta a}$), 2.98 (3H, s, Tyr^3-NMe), 3.01—3.10 (1H, m, Tyr^6-$H_{\beta b}$), 3.12 (3H, s, Tyr^5-NMe), 3.30 (1H, dd, J=14.3, 11.0 Hz, Tyr^3-$H_{\beta a}$), 3.44 (1H, dd, J=14.3, 4.4 Hz, Tyr^3-$H_{\beta b}$), 3.63 (1H, dd, J=11.0, 4.4 Hz, Tyr^3-H_α), 3.66 (1H, t, J=11.3 Hz, Tyr^5-$H_{\beta b}$), 3.78 (3H, s, Tyr^3-OMe), 3.93 (3H, s, Tyr^6-OMe), 4.34 (1H, d, J=1.9 Hz, Tyr^6-$H_{\delta a}$), 4.38 (1H, dd, J=7.0, 6.8 Hz, Ala^1-H_α), 4.54 (1H, dd, J=12.3, 4.2 Hz, Tyr^6-H_α), 4.73 (1H, dq, J=7.4, 6.6 Hz, Ala^4-H_α), 4.97 (1H, dd, J=15.9, 7.5 Hz, Met^2-H_α), 5.39 (1H, dd, J=11.3, 3.1 Hz, Tyr^5-H_α), 6.41 (1H, d, J=6.8 Hz, Ala^1-NH), 6.52 (1H, d, J=8.7 Hz, Met^2-NH), 6.57 (1H, dd, J=8.4, 1.9 Hz, Tyr^6-$H_{\delta b}$), 6.71 (1H, d, J=7.4 Hz, Ala^4-NH), 6.80 (1H, d, J=8.4 Hz, Tyr^6-H_ε), 6.83 (2H, d, J=8.6 Hz, Tyr^3-H_ε), 6.87 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-$H_{\varepsilon a}$), 7.06 (2H, d, J=8.6 Hz, Tyr^3-H_δ), 7.21 (1H, dd, J=8.4, 2.4 Hz, Tyr^5-$H_{\varepsilon b}$), 7.26 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-$H_{\delta a}$), 7.42 (1H, dd, J=8.4, 2.2 Hz, Tyr^5-$H_{\delta b}$).

Cell Survival by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide] Assay MTT colorimetric assay was performed in a 96-well plate.[12] The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Human KB oral epidermoid carcinoma cells (1×10^4 cells/ml) or mouse P388 leukemia cells (2×10^4 cells/ml) were inoculated in each well with 100 μl of RPMI1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Flow Laboratories, U.K.), 100 units/ml of penicillin and 100 μg/ml of streptomycin. After overnight incubation (37 °C, 5% CO_2), 100 μl of sample solution was added to each well and the plates were incubated for 3 d (KB Cell) or 2 d (P388). Then 50 μl of MTT (200 μg/ml PBS) was added to each well and the plates were incubated for a further 4 h. The resulting formazan was dissolved in 150 μl of DMSO. The plates was placed on a plate shaker for 5 min and read immediately at 540 nm. The IC_{50} (μg/ml) value was defined as that concentration of sample which caused 50% reduction of growth in sample-treated cells, with respect to the controls. The IC_{50} was calculated by using the probit test.

***In Vivo* Antitumor Activity** P388 murine leukemia cells (1×10^6 cells)

were inoculated i.p. into female CDF_1 mice (6—7 weeks old, control: $n=16$; test: $n=8$) on day 0. Samples, suspended in 0.5% gum arabic-saline solution, were administered i.p. on days 1—5. The antitumor activity was estimated according to the NCI tumor panel screening method.[13]

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