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Structures of cytotoxic bicyclic hexapeptides, RA-XIX, -XX, -XXI, and -XXII, from *Rubia cordifolia* L.

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Abstract

Novel bicyclic hexapeptides, RA-XIX, -XX, -XXI, and -XXII, were isolated from the roots of *Rubia cordifolia* L. The structures of RA-XIX and RA-XX were established by semisynthesis from a cycloisodityrosine, derived from previously reported RA-VII, and those of RA-XXI and RA-XXII by chemical correlation with RA-XX and previously reported RA-VIII, respectively. The IC_{50} values of these new peptides against P-388 leukemia cells were 0.013–0.63 μg/mL.

Keywords: RA-XIX; RA-XX; RA-XXI; RA-XXII; *Rubia cordifolia*; Bouvardin; Cytotoxicity

1. Introduction

RA-VII (**1**)[1,2] and bouvardin (NSC 259968, **2**)[3] are tumor cell growth inhibitors isolated from the plants, *Rubia cordifolia* L. (Rubiaceae) and *Bouvardia ternifolia* (Cav.) Schltdl. (Rubiaceae), respectively. Their structures are characterized by the presence of a 14-membered strained ring, an unusual cycloisodityrosine unit, formed by an ether linkage between the two aromatic rings of Tyr-5 and Tyr-6. Their antitumor action is considered to be due to inhibition of protein synthesis through interaction with eukaryotic ribosomes.[4,5] Recently, RA-VII (**1**) was shown to cause conformational changes in F-actin and stabilization of actin filaments to induce G2 arrest.[6] In our further studies on the cyclopeptide constituents in the roots of *R. cordifolia*, four new RA-series peptides, RA-XIX (**3**), -XX (**4**), -XXI (**5**), and -XXII (**6**), were isolated. This paper describes their isolation, structure determination, and cytotoxicity against P-388 cells.

2. Results and discussion

A methanol extract obtained from dried roots of *R. cordifolia* (50 kg) was partitioned between chloroform and water. The chloroform-soluble portion was subjected to a series of column chromatography using silica gel, alumina, and then aminopropyl-bonded silica gel eluting with a series of chloroform/methanol mixtures to give a fraction rich in RAs. The residue of this fraction, obtained after removal of the solvent, was crystallized from methanol to give crystals of crude RAs, which, after reversed-phase HPLC (ODS), afforded RA-XIX (**3**, 2.6 mg, 5.2×10^{-6}%) and RA-XX (**4**, 4.6 mg, 9.2×10^{-6}%). The mother liquor from the crystallization gave RA-XXI (**5**, 5.5 mg, 1.1×10^{-5}%) and RA-XXII (**6**, 34.5 mg, 6.9×10^{-5}%).

RA-XIX (**3**) was obtained as an amorphous solid. Its molecular formula was determined to be $C_{44}H_{56}N_6O_9$ from the $[M+H]^+$ peak at *m/z* 813.4187 (calcd for $C_{44}H_{57}N_6O_9$, 813.4187) in the HRESIMS. The ^1H and ^{13}C NMR spectra of **3** in $CDCl_3$ (Tables 1 and 2) showed signals typical of an

Abbreviations: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DPPA, diphenylphosphoryl azide; EDC·HCl, *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; HOOBt, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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Table 1
^{1}H NMR data for the major conformers of RA-XIX (**3**), -XX (**4**), -XXI (**5**), and -XXII (**6**) in $CDCl_3$ at 300 K[a]

Position		RA-XIX (**3**)	RA-XX (**4**)	RA-XXI (**5**)	RA-XXII (**6**)
		δ_H	δ_H	δ_H	δ_H
D-Ala-1	α	4.34 (qd, 6.9, 6.8)	4.35 (qd, 7.0, 6.8)	4.37 (qd, 7.0, 6.9)	4.47 (septet, 7.0)
	β	1.30 (d, 6.9, 3H)	1.31 (d, 7.0, 3H)	1.31 (d, 7.0, 3H)	1.35 (d, 7.0, 3H)
	NH	6.39 (d, 6.8)	6.40 (d, 6.8)	6.40 (d, 6.9)	6.38 (d, 7.0)
AA-2	α	4.85 (m)	4.71 (m)	4.70 (m)	4.44 (dd, 7.9, 1.2)
	β	1.52–1.66 (m, 2H)	1.67–1.84 (m, 2H)	1.67–1.84 (m, 2H)	4.09 (qd, 6.3, 1.2)
	γ	1.61 (m)	0.96 (t, 7.4, 3H)	0.96 (t, 7.4, 3H)	1.21 (d, 6.3, 3H)
	δa	0.94 (d, 6.1, 3H)			
	δb	0.93 (d, 6.1, 3H)			
	NH	6.04 (d, 8.8)	6.15 (d, 8.9)	6.16 (d, 8.7)	6.60 (d, 7.9)
Tyr-3	α	3.60 (dd, 11.1, 4.2)	3.62 (dd, 11.1, 4.2)	3.62 (dd, 11.1, 4.2)	3.64 (dd, 10.7, 5.0)
	βa	3.44 (dd, 14.2, 4.2)	3.46 (dd, 14.2, 4.2)	3.46 (dd, 14.2, 4.2)	3.39 (dd, 14.1, 5.0)
	βb	3.32 (dd, 14.2, 11.1)	3.31 (dd, 14.2, 11.1)	3.31 (dd, 14.2, 11.1)	3.35 (dd, 14.1, 10.7)
	δ	7.07 (d-like, 8.6, 2H)	7.08 (d-like, 8.6, 2H)	7.08 (d-like, 8.6, 2H)	7.07 (d-like, 8.6, 2H)
	ε	6.83 (d-like, 8.6, 2H)	6.83 (d-like, 8.6, 2H)	6.83 (d-like, 8.6, 2H)	6.86 (d-like, 8.6, 2H)
	NMe	2.92 (s, 3H)	2.92 (s, 3H)	2.93 (s, 3H)	2.98 (s, 3H)
	OMe	3.80 (s, 3H)	3.79 (s, 3H)	3.79 (s, 3H)	3.80 (s, 3H)
Ala-4	α	4.72 (dq, 7.5, 6.7)	4.73 (dq, 7.4, 6.7)	4.74 (dq, 7.5, 6.7)	4.75 (dq, 7.6, 6.7)
	β	1.14 (d, 6.7, 3H)	1.14 (d, 6.7, 3H)	1.13 (d, 6.7, 3H)	1.10 (d, 6.7, 3H)
	NH	6.68 (d, 7.5)	6.69 (d, 7.4)	6.68 (d, 7.5)	6.71 (d, 7.6)
Tyr-5	α	5.40 (dd, 11.3, 3.0)	5.40 (dd, 11.4, 3.1)	5.39 (dd, 11.4, 3.0)	5.40 (dd, 11.3, 3.0)
	βa	2.63 (dd, 11.3, 3.0)	2.63 (dd, 11.4, 3.1)	2.63 (dd, 11.4, 3.0)	2.63 (dd, 11.3, 3.0)
	βb	3.67 (t, 11.3)	3.67 (t, 11.4)	3.68 (t, 11.4)	3.70 (t, 11.3)
	δa	7.27 (dd, 8.4, 2.2)	7.27 (dd, 8.4, 2.2)	7.28 (dd, 8.5, 2.2)	7.28 (dd, 8.4, 2.2)
	δb	7.42 (dd, 8.4, 2.2)	7.42 (dd, 8.4, 2.2)	7.43 (dd, 8.4, 2.2)	7.41 (dd, 8.4, 2.2)
	εa	6.88 (dd, 8.4, 2.4)	6.88 (dd, 8.4, 2.4)	6.84 (dd, 8.5, 2.4)	6.84 (dd, 8.4, 2.4)
	εb	7.21 (dd, 8.4, 2.4)	7.21 (dd, 8.4, 2.4)	7.21 (dd, 8.4, 2.4)	7.20 (dd, 8.4, 2.4)
	NMe	3.13 (s, 3H)	3.13 (s, 3H)	3.12 (s, 3H)	3.11 (s, 3H)
Tyr-6	α	4.55 (dd, 12.0, 3.8)	4.54 (dd, 12.1, 3.9)	4.54 (dd, 12.0, 3.9)	4.57 (dd, 12.0, 3.9)
	βa	3.10 (dd, 18.0, 12.0)	3.09 (dd, 18.1, 12.1)	3.05 (dd, 17.8, 12.0)	3.06 (dd, 18.1, 12.0)
	βb	2.96 (dd, 18.0, 3.8)	2.96 (dd, 18.1, 3.9)	2.94 (dd, 17.8, 3.9)	2.94 (dd, 18.1, 3.9)
	δa	6.58 (dd, 8.3, 2.0)	6.58 (dd, 8.3, 2.0)	6.52 (dd, 8.2, 2.0)	6.53 (dd, 8.2, 1.9)
	δb	4.35 (d, 2.0)	4.34 (d, 2.0)	4.35 (d, 2.0)	4.35 (d, 1.9)
	εa	6.80 (d, 8.3)	6.80 (d, 8.3)	6.82 (d, 8.2)	6.82 (d, 8.2)
	NMe	2.68 (s, 3H)	2.68 (s, 3H)	2.67 (s, 3H)	2.69 (s, 3H)
	OMe	3.94 (s, 3H)	3.94 (s, 3H)		
	OH			5.66 (s)	5.66 (s)

[a] Recorded at 500 MHz, chemical shifts referenced to residual C*H*Cl_3 (7.26 ppm); *J*-values given in Hz are in parentheses.

RA-series peptide, and also demonstrated the presence of two conformers in a ratio of 87:13.[7,8] The structure of **3** was determined using the resonances caused by the major conformer, which included signals for four secondary methyl groups (δ_H/δ_C 0.93/22.9, 0.94/22.5, 1.14/18.6, 1.30/20.9), three *N*-methyl groups (δ_H/δ_C 2.68/29.2, 2.92/39.8, 3.13/30.5), two *O*-methyl groups (δ_H/δ_C 3.80/55.3, 3.94/56.2), four methylenes (δ_H/δ_C 1.52–1.66/40.3; 2.63, 3.67/37.1; 2.96, 3.10/35.4; 3.32, 3.44/32.9), one methine (δ_H/δ_C 1.61/24.8), six *N*-substituted methines (δ_H/δ_C 3.60/68.6, 4.34/48.0, 4.55/57.5, 4.72/46.4, 4.85/47.4, 5.40/54.2), two 1,4-disubstituted benzene rings (δ_H/δ_C 6.83/114.0×2, 7.07/130.3×2, δ_C 130.8, 158.4; δ_H/δ_C 6.88/124.3, 7.21/125.9, 7.27/132.8, 7.42/131.1, δ_C 135.1, 158.3), one 1,2,4-trisubstituted benzene ring (δ_H/δ_C 4.35/113.4, 6.58/120.9, 6.80/112.3, δ_C 128.2, 146.5, 153.1), six amide carbonyl groups (δ_C 167.8, 169.5, 170.6, 171.8, 172.1, 172.2), and three amide protons (δ_H 6.04, 6.39, 6.68). The analysis of its ^{1}H–^{1}H COSY and HMBC spectra revealed the features of the component amino acid units and the amino acid sequence of **3** (Fig. 2). In **3** the C-α (δ_C 47.4) of amino acid at position-2 (AA-2) was connected to the methylene carbon (C-β, δ_C 40.3), which was further connected to the methine (C-γ, δ_C 24.8) of an isopropyl unit, thus indicating that peptide **3** had a leucine unit as AA-2. The profiles of the ^{1}H and ^{13}C NMR chemical shifts due to the peptide backbone of **1** and **3** were very similar, indicating that they shared the same relative configuration. These observations implied that peptide **3** was an analogue of **1** whose Ala-2 was replaced by leucine, which was confirmed by the semisynthesis of **3** as shown in Scheme 1. After N-deprotection by catalytic hydrogenolysis, dipeptide **7**[9] was coupled with Boc-Leu-OH using PyBOP to afford tripeptide **8a**. Peptide **8a** was further treated with Cbz-D-Ala-OH to produce tetrapeptide **9a**, which on treatment with lithium hydroperoxide gave tetrapeptide acid **10a**. After deprotection of the N-terminus of cycloisodityrosine **11**, prepared by degradation of natural **1**,[9,10] **11** was coupled with acid **10a** to afford hexapeptide **12a**. Deprotection and subsequent macrocyclization of **12a** using DPPA gave a product that was shown to be identical to natural **3** by comparison of their spectroscopic data and optical rotations. Thus,

Table 2
^{13}C NMR data for the major conformers of RA-XIX (**3**), -XX (**4**), -XXI (**5**), and -XXII (**6**) in $CDCl_3$ at 300 K[a]

Position		RA-XIX (**3**)	RA-XX (**4**)	RA-XXI (**5**)	RA-XXII (**6**)
		δ_C	δ_C	δ_C	δ_C
D-Ala-1	α	48.0	48.1	48.0	47.8
	β	20.9	20.9	20.9	21.0
	C=O	172.2	172.3	172.4	172.9
AA-2	α	47.4	50.3	50.4	51.2
	β	40.3	24.7	24.6	66.1
	γ	24.8	10.1	10.2	19.3
	δa	22.5			
	δb	22.9			
	C=O	172.1	172.0	172.1	172.3
Tyr-3	α	68.6	68.7	68.7	68.5
	β	32.9	33.0	33.0	32.8
	γ	130.8	130.9	130.8	130.4
	δ	130.3[b]	130.2[b]	130.2[b]	130.1[b]
	ε	114.0[b]	114.1[b]	114.1[b]	114.2[b]
	ζ	158.4	158.4	158.4	158.5
	C=O	167.8	167.8	167.9	167.6
	NMe	39.8	39.8	39.8	40.3
	OMe	55.3	55.3	55.3	55.3
Ala-4	α	46.4	46.4	46.4	46.2
	β	18.6	18.6	18.6	18.6
	C=O	171.8	171.8	171.8	171.6
Tyr-5	α	54.2	54.2	54.2	54.4
	β	37.1	37.0	37.0	36.9
	γ	135.1	135.1	135.6	135.7
	δa	132.8	132.8	133.0	133.0
	δb	131.1	131.0	131.1	131.0
	εa	124.3	124.3	124.2	124.2
	εb	125.9	125.9	125.9	125.9
	ζ	158.3	158.3	157.9	157.9
	C=O	169.5	169.4	169.2	169.1
	NMe	30.5	30.5	30.5	30.5
Tyr-6	α	57.5	57.4	57.5	57.5
	β	35.4	35.4	35.5	35.6
	γ	128.2	128.1	127.6	127.6
	δa	120.9	120.9	121.6	121.7
	δb	113.4	113.4	113.0	113.0
	εa	112.3	112.3	115.7	115.7
	εb	153.1	153.1	151.1	151.0
	ζ	146.5	146.5	143.0	143.0
	C=O	170.6	170.6	170.5	170.6
	NMe	29.2	29.2	29.3	29.4
	OMe	56.2	56.2		

[a] Recorded at 125 MHz, chemical shifts referenced to *C*DCl_3 (77.03 ppm).
[b] Two carbons.

the absolute structure of RA-XIX was determined to be as shown in structure **3** (Fig. 1).

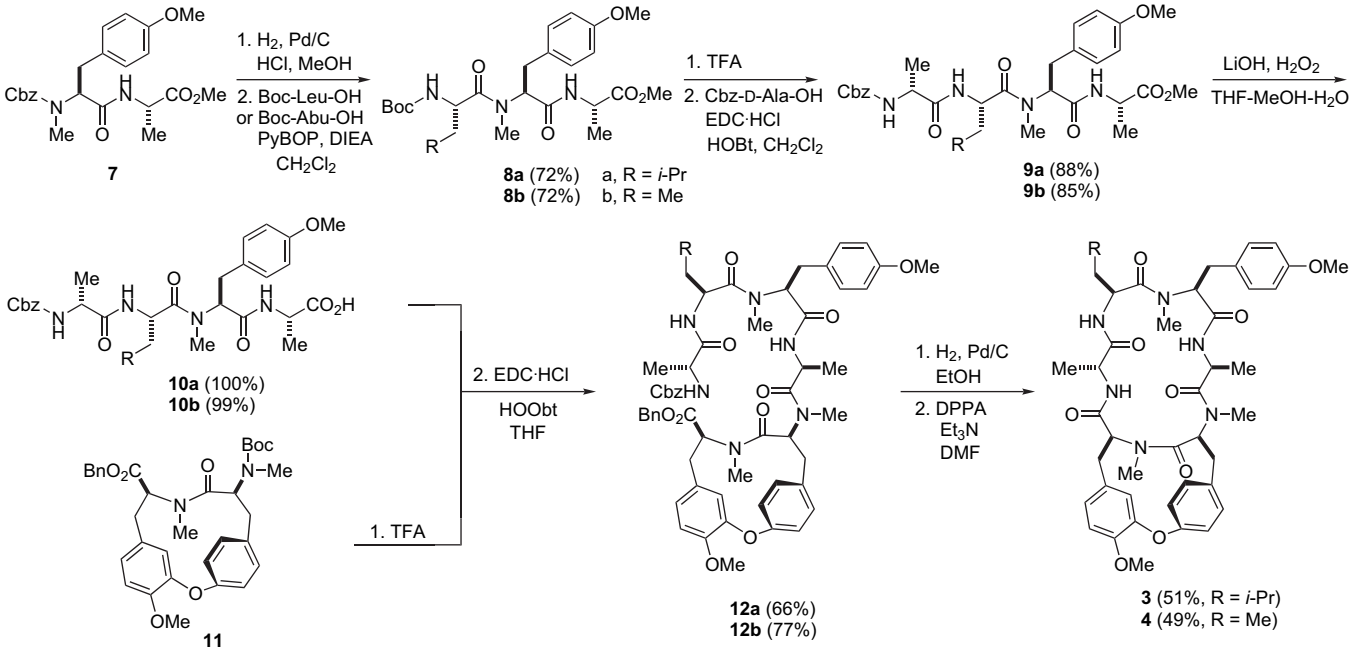
RA-XX (**4**) was obtained as an amorphous solid. Its molecular formula was determined to be $C_{42}H_{52}N_6O_9$ from the $[M+H]^+$ peak at *m/z* 785.3879 (calcd for $C_{42}H_{53}N_6O_9$, 785.3874) in the HRESIMS. Its ^{1}H and ^{13}C NMR spectra were very similar to those of RA-XIX (**3**) except for the resonances due to those of AA-2. In the ^{1}H NMR spectrum of **4**, a characteristic triplet methyl signal (δ_H 0.96, t, J=7.4 Hz) was observed, and analysis of its ^{1}H–^{1}H COSY and HMBC spectra revealed the presence of a CH_3–CH_2–CH(NH–C=O)–C=O unit (Fig. 3). Accordingly, peptide **4** was considered to be an analogue of **3** whose Leu-2 was replaced by 2-aminobutyric acid, which was verified by semisynthesis of **4** through a similar protocol employed in the synthesis of **3** (Scheme 1). Thus, tetrapeptide acid **10b** with a 2-aminobutyric acid was prepared from dipeptide **7**[9] and then converted into hexapeptide **12b**, which, after deprotection, was subjected to macrocyclization to give a product that was shown to be identical to natural **4** by comparison of their spectroscopic data and optical rotations. Thus, the absolute structure of RA-XX was determined to be as shown in structure **4** (Fig. 1).

RA-XXI (**5**) was obtained as a white crystalline powder. Its molecular formula was determined to be $C_{41}H_{50}N_6O_9$ from the $[M+Na]^+$ peak at *m/z* 793.3495 (calcd for $C_{41}H_{50}N_6O_9Na$, 793.3537) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **5** were very similar to those of **4**, except that in the ^{1}H NMR spectrum of **5** one methoxyl signal was missing and instead had a phenolic hydroxyl signal at δ_H 5.66. This phenolic hydroxyl signal showed cross-peaks with C-εa, C-εb, and C-ζ in the HMBC spectrum, thus indicating that in **5** a hydroxyl group substituted for the methoxyl group at the ζ-position of Tyr-6 in **4**. A downfield shift of the ^{13}C NMR signal for C-εa in Tyr-6 from δ_C 112.3 for **4** to δ_C 115.7 for **5** also explains the replacement of the methoxyl group by a hydroxyl group. Treatment of **5** with (trimethylsilyl)diazomethane afforded a product that was shown to be identical to natural **4** from their spectroscopic data and optical rotations. Thus, the absolute structure of RA-XXI was determined to be as shown in structure **5** (Fig. 1).

RA-XXII (**6**) was obtained as an amorphous solid. Its molecular formula was determined to be $C_{41}H_{50}N_6O_{10}$ from the $[M+Na]^+$ peak at *m/z* 809.3461 (calcd for $C_{41}H_{50}N_6O_{10}Na$, 809.3486) in the HRESIMS. Its ^{1}H NMR spectrum bore a close resemblance to that of RA-VIII (**13**),[11] but the signal for the *O*-methyl group at Tyr-6 was missing. The presence of a phenolic hydroxyl signal at δ_H 5.66 and close similarity of the ^{13}C NMR chemical shifts for the aromatic carbons of Tyr-6 in **6** with those in **5** indicated that the methoxyl group at Tyr-6 in **13** was replaced by a hydroxyl group in **6**. Treatment of **6** with (trimethylsilyl)diazomethane afforded a product that was shown to be identical to natural **13** from their spectroscopic data and optical rotations. Since the absolute structure of **13** has already been established,[11] that of RA-XXII was determined to be as shown in structure **6** (Fig. 1).

Peptides **3**–**6** were evaluated for their cytotoxicity against P-388 murine leukemia cells, with peptide **1** as reference. The results are summarized in Table 3. For the peptides having a methoxyl group in Tyr-6, the order of cytotoxicity was **1**>**4**>**3**. Peptide **6**, having a hydroxyl group in its Thr-2, was less cytotoxic than **5**. This is consistent with our earlier observations that the cytotoxicity decreases with increase in the length of the carbon side chain or introduction of a polar functionality at this location.[12]

The representative RA-series peptides, RA-VII (**1**) and bouvardin (**2**), are composed of one D-alanine (D-Ala-1), two L-alanines (Ala-2, Ala-4), one *N*,*O*-dimethyl-L-tyrosine (Tyr-3), and one modified *N*,*N*′-dimethyl-L,L-cycloisodityrosine (Tyr-5, Tyr-6). In some RA-series peptides, Ala-2 of **1** was replaced by L-serine (RA-III),[13] L-threonine (RA-VIII, **13**),[11]



Scheme 1. Synthesis of RA-XIX (**3**) and RA-XX (**4**).

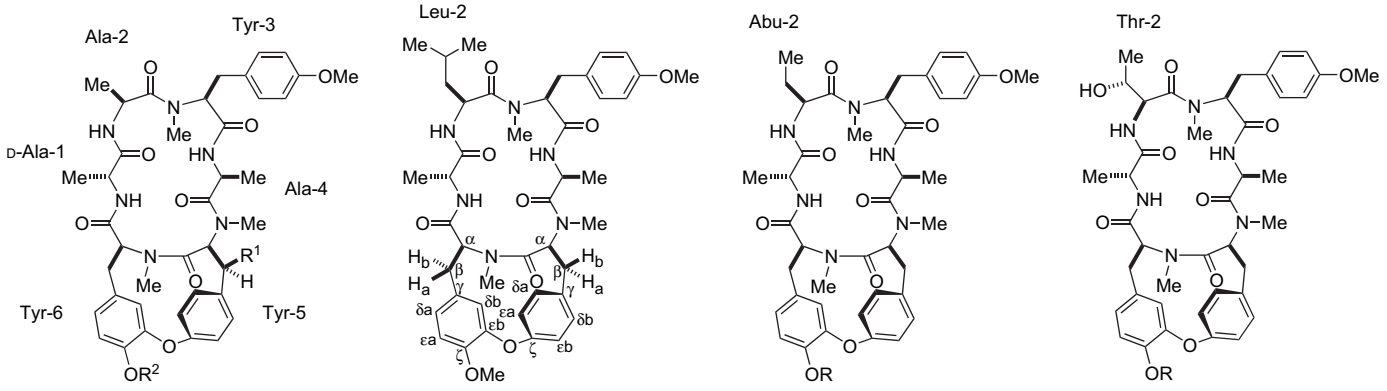


Figure 1.

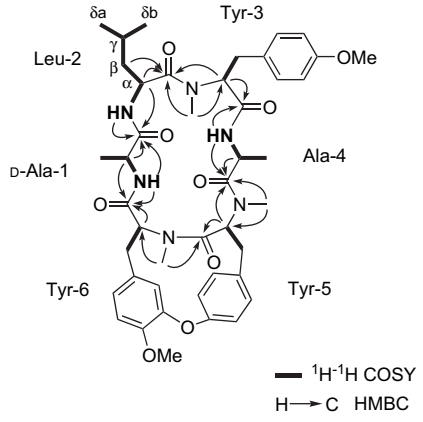


Figure 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations for determination of the peptide sequence of **3**.

L-pyroglutamic acid (RA-IX), or L-glutamic acid (RA-X).[14] RA-XIX (**3**) is the first example of RA-series peptides incorporating L-leucine, whereas RA-XX (**4**) and RA-XXI (**5**) are also the first examples of this series of peptides incorporating L-2-aminobutyric acid, and RA-XXII (**6**) is the second example having L-threonine.

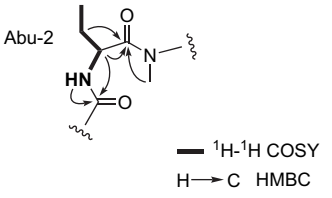


Figure 3. Partial structure and selected $^{1}H-^{1}H$ COSY and HMBC correlations for **4**.

Table 3
Cytotoxicity of RA-VII (**1**), RA-XIX (**3**), RA-XX (**4**), RA-XXI (**5**), and RA-XXII (**6**) against P-388 leukemia cells

Compound	IC_{50} (μg/mL)
1	0.0023
3	0.024
4	0.013
5	0.041
6	0.63

3. Experimental

3.1. General

Melting points were determined on a Yanaco MP-3 apparatus and are recorded uncorrected. Optical rotations were measured on a JASCO P-1030 digital polarimeter, IR spectra on a JASCO FT/IR 620 spectrophotometer, and UV spectra on a JASCO V-530 spectrophotometer. NMR spectra were measured on a Bruker DRX-500 spectrometer at 300 K. The ^{1}H chemical shifts in $CDCl_3$ or CD_3OD were referenced to the residual $CHCl_3$ (7.26 ppm) or CD_2HOD (3.31 ppm), and the ^{13}C chemical shifts were referenced to the solvent ($CDCl_3$, 77.03 ppm; CD_3OD, 49.0 ppm). Mass spectra were obtained with a Micromass LCT spectrometer. Preparative HPLC was carried out on a Shimadzu LC-6AD pump unit equipped with a SPD-10A UV detector (λ 254 nm) and a pre-packed ODS column (5 μm, 20×250 mm), using a MeOH/H_2O or a MeCN/H_2O solvent system at a flow rate of 10 mL/min.

3.2. Plant material

The roots of *R. cordifolia* L. were obtained from a market in Tokyo in March 2004. The material was identified by Prof. Koichi Takeya, and a voucher specimen (Tko-0403–01) has been deposited at the Herbarium of Tokyo University of Pharmacy and Life Sciences.

3.3. Extraction and isolation

The dried roots (50 kg) of *R. cordifolia* were extracted with MeOH (3×175 L). After removal of MeOH under reduced pressure, the residue (3.6 kg) was partitioned between chloroform and water. The chloroform-soluble portion (993 g) was placed on a column of silica gel (Merck, 70–230 mesh, 3.6 kg) and eluted with $CHCl_3$ (9 L), EtOAc (18 L), and $CHCl_3$/MeOH (9:1, 27 L), sequentially to give three fractions. After removal of the solvent, the residue of the $CHCl_3$/MeOH (9:1) fraction (152 g) was subjected to alumina (Merck, 3 kg) column chromatography (CC) eluting sequentially with $CHCl_3$ (2 L) and $CHCl_3$/MeOH (9:1, 12 L). After evaporation, the $CHCl_3$/MeOH (9:1) fraction (41.9 g) was subjected to aminopropyl-bonded silica gel (Chromatorex, 200–350 mesh, 300 g) CC eluting sequentially with $CHCl_3$ (6 L) and $CHCl_3$/MeOH (9:1, 1 L). The residue obtained after removal of the solvent of the $CHCl_3$ eluate was crystallized from methanol to give crystals of crude RAs (8.5 g) and mother liquor (ML). The crystals were then subjected to ODS HPLC using MeOH/H_2O (60:40, then 100:0) to give four fractions, C1 (4.42 g, mostly deoxybouvardin), C2 (0.32 g), C3 (3.44 g, mostly RA-VII), and C4 (0.23 g, MeOH eluate). Fraction C4 was separated by repeated ODS HPLC using MeCN/H_2O (37:63 and 45:55) to afford RA-XIX (**3**, 2.6 mg, 5.2×10^{-6}%) and RA-XX (**4**, 4.6 mg, 9.2×10^{-6}%).

After removal of the solvent, ML (10.2 g) was subjected to ODS HPLC using MeOH/H_2O (60:40, then 100:0) to give five fractions, M1 (0.42 g), M2 (2.44 g, mostly deoxybouvardin), M3 (0.33 g), M4 (0.11 g, mostly RA-VII), and M5 (1.12 g). Fraction M1 was separated by repeated ODS HPLC using MeCN/H_2O (33:67) and then MeOH/H_2O (55:45) to afford RA-XXII (**6**, 34.5 mg, 6.9×10^{-5}%). Separation of fraction M3 by ODS HPLC using MeCN/H_2O (30:70) yielded RA-XXI (**5**, 5.5 mg, 1.1×10^{-5}%).

3.4. Characteristics of each peptide

3.4.1. RA-XIX (*3*)

Amorphous solid; $[\alpha]_D^{26}$ −224.4 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3390, 2954, 2932, 1655, 1635, 1513, 1413, 1265, 1128, 1032, 802 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.81), 225sh (4.54), 277 (3.74) nm; ^{1}H and ^{13}C NMR, a mixture of two conformers in a ratio of 87:13 in $CDCl_3$ at 300 K. For the data of the major conformer, refer to Tables 1 and 2; HRESIMS *m/z* 813.4187 ([M+H]$^+$, calcd for $C_{44}H_{57}N_6O_9$, 813.4187).

3.4.2. RA-XX (*4*)

Amorphous solid; $[\alpha]_D^{26}$ −218.4 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 3388, 2934, 1654, 1635, 1513, 1414, 1265, 1128, 1031, 752 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.77), 225sh (4.48), 277 (3.61) nm; ^{1}H and ^{13}C NMR, a mixture of two conformers in a ratio of 89:11 in $CDCl_3$ at 300 K. For the data of the major conformer, refer to Tables 1 and 2; HRESIMS *m/z* 785.3879 ([M+H]$^+$, calcd for $C_{42}H_{53}N_6O_9$, 785.3874).

3.4.3. RA-XXI (*5*)

White crystalline powder, mp>300 °C; $[\alpha]_D^{26}$ −230.1 (*c* 0.28, $CHCl_3$); IR (film) ν_{max} 3386, 2930, 1655, 1633, 1513, 1413, 1247, 1094, 1035, 754 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.77), 226sh (4.49), 278 (3.61) nm; ^{1}H and ^{13}C NMR, a mixture of two conformers in a ratio of 87:13 in $CDCl_3$ at 300 K. For the data of the major conformer, refer to Tables 1 and 2; HRESIMS *m/z* 793.3495 ([M+Na]$^+$, calcd for $C_{41}H_{50}N_6O_9Na$, 793.3537).

3.4.4. RA-XXII (*6*)

Amorphous solid; $[\alpha]_D^{26}$ −186.7 (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3387, 2934, 1658, 1629, 1513, 1412, 1247, 1094, 1034, 752 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.76), 225sh (4.47), 277 (3.61) nm; ^{1}H and ^{13}C NMR, a mixture of three conformers in a ratio of 73:25:2 in $CDCl_3$ at 300 K. For the data of the most populated conformer, refer to Tables 1 and 2; HRESIMS *m/z* 809.3461 ([M+Na]$^+$, calcd for $C_{41}H_{50}N_6O_{10}Na$, 809.3486).

3.5. Semisynthesis of RA-XIX (**3**)

3.5.1. Boc-Leu-N,O-dimethyl-Tyr-Ala-OMe (**8a**)

Palladium (10%) on charcoal catalyst (5 mg) and hydrochloric acid (0.1 mL) were added to a solution of Cbz-*N*,*O*-dimethyl-Tyr-Ala-OMe (**7**)[9] (31.8 mg, 0.0742 mmol) in MeOH (2 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 2 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (2 mL) together with Boc-Leu-OH (34.3 mg, 0.148 mmol) and PyBOP (77.2 mg, 0.148 mmol), to which DIEA (52.9 μL, 0.304 mmol) was slowly added at −20 °C under an atmosphere of argon. The mixture was stirred at this temperature for 1 h, and then at room temperature for 5 days. Aqueous citric acid (10%, 2 mL) was added to the mixture, and the whole was extracted with $CHCl_3$ (3×7 mL). The combined $CHCl_3$ extracts were washed sequentially with saturated aqueous $NaHCO_3$ (2 mL) and brine (2 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 70:30) to afford **8a** (27.2 mg, 72%) as an amorphous solid. $[\alpha]_D^{27}$ −102.7 (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3299, 2956, 1747, 1681, 1636, 1513, 1456, 1366, 1259, 1173, 1036, 772 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, major conformer) δ 8.25 (d, 1H, *J*=7.0 Hz), 7.02 (d-like, 2H, *J*=8.5 Hz), 6.82 (d-like, 2H, *J*=8.5 Hz), 4.90 (d, 1H, *J*=7.4 Hz), 4.66 (dd, 1H, *J*=11.0, 3.5 Hz), 4.52 (m, 1H), 4.16 (m, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.18 (dd, 1H, *J*=14.7, 3.5 Hz), 2.98 (dd, 1H, *J*=14.7, 11.0 Hz), 2.90 (s, 3H), 1.46 (m, 1H), 1.40 (d, 3H, *J*=7.2 Hz), 1.39 (m, 1H), 1.37 (s, 9H), 1.16 (ddd, 1H, *J*=14.2, 11.9, 4.1 Hz), 0.66 (d, 3H, *J*=7.2 Hz), 0.64 (d, 3H, *J*=7.0 Hz), −0.22 (ddd, 1H, *J*=14.2, 11.0, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, major conformer) δ 174.1 (s), 173.1 (s), 169.1 (s), 158.6 (s), 156.6 (s), 130.5 (d, 2C), 129.6 (s), 114.5 (d, 2C), 80.4 (s), 62.5 (d), 55.1 (q), 52.2 (q), 48.6 (d), 47.9 (d), 38.4 (t), 33.0 (t), 29.3 (q), 28.2 (q, 3C), 24.0 (d), 22.9 (q), 20.2 (q), 17.4 (q); HRESIMS *m/z* 508.2992 ($[M+H]^+$, calcd for $C_{26}H_{42}N_3O_7$, 508.3023).

3.5.2. Cbz-D-Ala-Leu-N,O-dimethyl-Tyr-Ala-OMe (**9a**)

A solution of **8a** (24.6 mg, 0.0484 mmol) in TFA (0.6 mL) was stirred at room temperature for 2 h. TFA was removed in vacuo and the residue was dissolved in $CHCl_3$ (3 mL). The solution was washed sequentially with saturated aqueous $NaHCO_3$ (3 mL) and brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue, Cbz-D-Ala-OH (16.2 mg, 0.0725 mmol), and HOBt (9.8 mg, 0.073 mmol) were dissolved in CH_2Cl_2 (1 mL), to which EDC·HCl (13.9 mg, 0.0725 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1 h, and then at room temperature for 3 days. Saturated aqueous $NaHCO_3$ (3 mL) was added to the solution and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 44:56) to afford **9a** (26.2 mg, 88%) as an amorphous solid. $[\alpha]_D^{28}$ −82.0 (*c* 0.26, $CHCl_3$); IR (film) ν_{max} 3288, 2955, 1724, 1627, 1514, 1455, 1248, 1035, 753 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, major conformer) δ 8.15 (br d, 1H, *J*=6.0 Hz), 7.36−7.28 (m, 5H), 7.10 (br d, 1H, *J*=6.2 Hz), 7.01 (d-like, 2H, *J*=8.6 Hz), 6.82 (d-like, 2H, *J*=8.6 Hz), 5.74 (d, 1H, *J*=7.8 Hz), 5.05 (s, 2H), 4.86 (m, 1H), 4.48 (quintet, 1H, *J*=7.1 Hz), 4.39 (m, 1H), 4.32 (m, 1H), 3.75 (s, 3H), 3.66 (s, 3H), 3.10 (dd, 1H, *J*=14.7, 3.5 Hz), 3.00 (dd, 1H, *J*=14.7, 11.1 Hz), 2.89 (s, 3H), 1.37 (m, 1H), 1.34 (d, 3H, *J*=7.1 Hz), 1.33 (m, 1H), 1.31 (d, 3H, *J*=7.2 Hz), 0.66 (br d, 3H, *J*=5.7 Hz), 0.58 (d, 3H, *J*=5.6 Hz), −0.09 (br t, 1H, *J*=11.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, major conformer) δ 173.5 (s), 173.3 (s), 173.1 (s), 168.7 (s), 158.6 (s), 155.7 (s), 136.2 (s), 130.5 (d, 2C), 129.4 (s), 128.5 (d, 2C), 128.2 (d), 128.1 (d, 2C), 114.5 (d, 2C), 67.0 (t), 62.4 (d), 55.1 (q), 52.3 (q), 50.2 (d), 48.5 (d), 46.9 (d), 38.2 (t), 33.2 (t), 29.3 (q), 24.3 (d), 23.0 (q), 20.3 (q), 19.0 (q), 17.5 (q); HRESIMS *m/z* 613.3210 ($[M+H]^+$, calcd for $C_{32}H_{45}N_4O_8$, 613.3237).

3.5.3. Cbz-D-Ala-Leu-N,O-dimethyl-Tyr-Ala-OH (**10a**)

A mixture of a LiOH solution [LiOH·H_2O (4.6 mg, 0.11 mmol) in H_2O (0.2 mL)] and aqueous H_2O_2 (35%, 0.1 mL) was slowly added to a cooled (0 °C) solution of **9a** (22.3 mg, 0.0364 mmol) in a mixture of THF/MeOH (1:1, 2 mL). The solution was stirred at 0 °C for 30 min and then at room temperature for 4 h. Aqueous $NaHSO_3$ (5%, 0.4 mL) and aqueous citric acid (10%, 0.8 mL) were added to the solution at 0 °C. After stirring for 20 min, the mixture was extracted with $CHCl_3$ (3×10 mL). The combined $CHCl_3$ extracts were washed with brine (5 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 5:1) to afford **10a** (21.7 mg, 100%) as colorless needles. Mp 98−102 °C (MeOH); $[\alpha]_D^{28}$ −99.0 (*c* 0.16, $CHCl_3$); IR (film) ν_{max} 3287, 2958, 1720, 1628, 1514, 1248, 1035, 755 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, major conformer) δ 7.37−7.27 (m, 5H), 7.11 (d-like, 2H, *J*=8.6 Hz), 6.87 (d-like, 2H, *J*=8.6 Hz), 5.06 (d, 1H, *J*=12.5 Hz), 5.03 (d, 1H, *J*=12.5 Hz), 4.93 (br d, 1H, *J*=11 Hz), 4.46 (dd, 1H, *J*=11.6, 2.8 Hz), 4.31 (q, 1H, *J*=7.2 Hz), 4.16 (q, 1H, *J*=7.2 Hz), 3.76 (s, 3H), 3.11 (dd, 1H, *J*=14.4, 2.5 Hz), 3.00 (dd, 1H, *J*=14.4, 11.3 Hz), 2.89 (s, 3H), 1.38 (d, 3H, *J*=7.2 Hz), 1.38 (m, 1H), 1.28 (m, 1H), 1.27 (d, 3H, *J*=7.2 Hz), 0.68 (d, 3H, *J*=6.5 Hz), 0.64 (d, 3H, *J*=6.4 Hz), −0.05 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD, major conformer) δ 178.0 (s), 175.5 (s), 175.3 (s), 171.0 (s), 160.2 (s), 157.9 (s), 138.1 (s), 131.7 (d, 2C), 130.8 (s), 129.5 (d, 2C), 129.1 (d, 2C), 128.9 (d), 115.5 (d, 2C), 67.6 (t), 63.9 (d), 55.6 (q), 51.6 (d), 50.7 (d), 48.3 (d), 39.5 (t), 34.2 (t), 30.2 (q), 25.4 (t), 23.5 (q), 20.9 (q), 18.8 (q), 17.9 (q); HRESIMS *m/z* 599.3079 ($[M+H]^+$, calcd for $C_{31}H_{43}N_4O_8$, 599.3081).

3.5.4. Hexapeptide **12a**

A solution of **11** (11.4 mg, 0.0198 mmol) in TFA (0.7 mL) was stirred at room temperature for 2 h. TFA was removed in

vacuo. $CHCl_3$ (5 mL) and saturated aqueous $NaHCO_3$ (5 mL) were added to the residue, and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was dissolved in THF (1 mL) together with **10a** (14.2 mg, 0.0237 mmol) and HOObt (6.5 mg, 0.040 mmol), to which EDC·HCl (7.6 mg, 0.040 mmol) was added at 0 °C. The mixture was stirred at this temperature for 1 h and then at room temperature for 48 h. Saturated aqueous $NaHCO_3$ (3 mL) was added to the residue and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 60:40) to afford **12a** (13.8 mg, 66%) as an amorphous solid. $[\alpha]_D^{26}$ −241.7 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3292, 2955, 1739, 1635, 1514, 1249, 1030, 752 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.89 (br m), 7.43−7.05 (m), 6.89 (dd, *J*=8.4, 2.5 Hz), 6.83 (d, *J*=8.6 Hz), 6.80 (d, *J*=8.7 Hz), 6.784 (d, *J*=8.4 Hz), 6.775 (d, *J*=8.4 Hz), 6.61 (dd, *J*=8.2, 2.1 Hz), 6.54 (m), 6.25 (br m), 5.86 (br m), 5.52 (br m), 5.37 (br m), 5.32 (dd, *J*=11.4, 3.3 Hz), 5.15−4.68 (m), 4.64 (dd, *J*=12.1, 3.6 Hz), 4.39 (m), 4.37 (d, *J*=2.0 Hz), 4.13 (br m), 3.96 (s), 3.931 (s), 3.926 (s), 3.76 (s), 3.75 (s), 3.74 (s), 3.64 (t, *J*=11.6 Hz), 3.61 (t, *J*=115 Hz), 3.31−3.14 (m), 3.27 (s), 3.18 (s), 3.05 (s), 3.01 (s), 2.98 (s), 3.00−2.93 (m), 2.96 (s), 2.90 (s), 2.88 (s), 2.85 (dd, *J*=9.4, 3.6 Hz), 2.79 (dd, *J*=11.4, 3.1 Hz), 2.72 (dd, *J*=11.4, 3.1 Hz), 2.66 (br s), 2.553 (s), 2.551 (s), 1.46 (br m), 1.34 (d, *J*=7.0 Hz), 1.30 (d, *J*=7.0 Hz), 1.26 (d, *J*=6.8 Hz), 1.25 (d, *J*=6.7 Hz), 1.18 (d, *J*=7.0 Hz), 0.93 (d, *J*=6.5 Hz), 0.87 (d, *J*=6.4 Hz), 0.79 (d, *J*=6.4 Hz), 0.67 (d, *J*=6.4 Hz), 0.58 (d, *J*=5.9 Hz); HRESIMS *m/z* 1055.5127 ($[M+H]^+$, calcd for $C_{59}H_{71}N_6O_{12}$, 1055.5130).

3.5.5. RA-XIX (3)

Palladium (10%) on charcoal catalyst (24 mg) was added to a solution of **12a** (12.0 mg, 0.0114 mmol) in EtOH (2 mL). Then, the reaction mixture was stirred at room temperature under an atmosphere of hydrogen for 2.5 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in DMF (11.4 mL). To this solution were added triethylamine (15.9 μL, 0.114 mmol) and DPPA (4.9 μL, 0.023 mmol) at 0 °C, and after stirring at room temperature for 3 days, the solvent was removed under reduced pressure. Saturated aqueous $NaHCO_3$ (10 mL) was added to the residue and the whole was extracted with $CHCl_3$ (3×10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was separated by HPLC (MeOH/H_2O 45:55) to give a compound [4.7 mg, 51%, $[\alpha]_D^{26}$ −212.8 (*c* 0.22, $CHCl_3$)], which was shown to be identical to natural **3** by comparison of their ^1H and ^{13}C NMR spectra, mass spectra, and optical rotations.

3.6. Semisynthesis of RA-XX (4)

3.6.1. Boc-Abu-N,O-dimethyl-Tyr-Ala-OMe (8b)

Palladium (10%) on charcoal catalyst (5 mg) and hydrochloric acid (0.1 mL) were added to a solution of Cbz-*N*,*O*-dimethyl-Tyr-Ala-OMe (**7**)[9] (32.3 mg, 0.0754 mmol) in MeOH (2 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 2 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue, Boc-Abu-OH (30.6 mg, 0.151 mmol), and PyBOP (78.5 mg, 0.150 mmol) were dissolved in CH_2Cl_2 (2 mL), to which DIEA (53.8 μL, 0.309 mmol) was slowly added at −20 °C under an atmosphere of argon. The mixture was stirred at −20 °C for 1 h and then at room temperature for 5 days. Aqueous citric acid (10%, 2 mL) was added to the mixture and the whole was extracted with $CHCl_3$ (3×7 mL). The combined $CHCl_3$ extracts were washed sequentially with saturated aqueous $NaHCO_3$ (2 mL) and brine (2 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 70:30) to afford **8b** (26.2 mg, 72%) as an amorphous solid. $[\alpha]_D^{28}$ −105.8 (*c* 0.37, $CHCl_3$), IR (film) ν_{max} 3425, 2978, 1642, 1515, 1249, 1174, 753 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, major conformer) δ 8.17 (d, 1H, *J*=7.2 Hz), 7.04 (d-like, 2H, *J*=8.5 Hz), 6.82 (d-like, 2H, *J*=8.5 Hz), 4.88 (d, 1H, *J*=7.2 Hz), 4.80 (dd, 1H, *J*=10.9, 3.7 Hz), 4.55 (quintet, 1H, *J*=7.3 Hz), 3.95 (m, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.16 (dd, 1H, *J*=14.6, 3.7 Hz), 2.97 (dd, 1H, *J*=14.6, 10.9 Hz), 2.88 (s, 3H), 1.38 (s, 9H), 1.38 (d, 3H, *J*=7.1 Hz), 1.03 (m, 1H), 0.62 (t, 3H, *J*=7.4 Hz), 0.12 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7 (s), 173.0 (s), 169.0 (s), 158.7 (s), 156.7 (s), 130.3 (d, 2C), 129.7 (s), 114.3 (d, 2C), 80.5 (s), 62.5 (d), 55.3 (q), 52.2 (q), 51.2 (d), 48.4 (d), 33.3 (t), 29.0 (q), 28.2 (q, 3C), 24.1 (t), 17.6 (q), 10.7 (q); HRESIMS *m/z* 502.2545 ($[M+Na]^+$, calcd for $C_{24}H_{37}N_3O_7Na$, 502.2529).

3.6.2. Cbz-D-Ala-Abu-N,O-dimethyl-Tyr-Ala-OMe (9b)

A solution of **8b** (30.0 mg, 0.0626 mmol) in TFA (0.6 mL) was stirred at room temperature for 2 h. TFA was removed in vacuo and the residue was dissolved in $CHCl_3$ (3 mL). The solution was washed sequentially with saturated aqueous $NaHCO_3$ (3 mL) and brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue, Cbz-D-Ala-OH (21.0 mg, 0.0941 mmol), and HOBt (12.7 mg, 0.0940 mmol) were dissolved in CH_2Cl_2 (1 mL), to which EDC·HCl (18.0 mg, 0.0939 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 3 days. Saturated aqueous $NaHCO_3$ (3 mL) was added to the solution, and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 45:55) to afford **9b** (31.2 mg, 85%) as an amorphous solid. $[\alpha]_D^{28}$ −82.1 (*c* 0.21, $CHCl_3$); IR (film) ν_{max} 3288, 2937, 1724, 1651, 1632, 1514, 1455, 1248, 1034, 754 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, major conformer) δ 8.06 (br d, 1H, *J*=6.0 Hz), 7.36−7.27 (m, 5H), 7.06 (m, 1H), 7.04 (d,

2H, J=8.6 Hz), 6.82 (d, 2H, J=8.6 Hz), 5.79 (d, 1H, J=7.6 Hz), 5.05 (s, 2H), 4.99 (br d, 1H, J=9.1 Hz), 4.48 (quintet, 1H, J=7.1 Hz), 4.31 (m, 1H), 4.12 (m, 1H), 3.75 (s, 3H), 3.65 (br s, 3H), 3.12 (dd, 1H, J=14.6, 3.6 Hz), 2.96 (dd, 1H, J=14.6, 11.1 Hz), 2.86 (s, 3H), 1.34 (br d, 3H, J=8.9 Hz), 1.31 (d, 3H, J=7.1 Hz), 1.20 (br m, 1H), 0.56 (br t, 3H, J=6.2 Hz), 0.26 (br m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, major conformer) δ 173.8 (s), 173.2 (s), 172.9 (s), 168.7 (s), 158.7 (s), 155.8 (s), 136.2 (s), 130.3 (d, 2C), 129.5 (d), 128.5 (d, 2C), 128.2 (d), 128.1 (d, 2C), 114.3 (d, 2C), 67.0 (t), 62.3 (d), 55.3 (q), 52.3 (q), 50.5 (d), 50.2 (d), 48.5 (d), 33.4 (t), 29.0 (q), 24.0 (t), 19.0 (q), 17.6 (q), 10.5 (q); HRESIMS m/z 585.2914 ($[M+H]^+$, calcd for $C_{30}H_{41}N_4O_8$, 585.2924).

3.6.3. *Cbz-D-Ala-Abu-N,O-dimethyl-Tyr-Ala-OH (**10b**)*

A mixture of a LiOH solution [$LiOH\cdot H_2O$ (5.9 mg, 0.14 mmol) in H_2O (0.2 mL)] and aqueous H_2O_2 (35%, 0.1 mL) was slowly added to a cooled (0 °C) solution of **9b** (27.2 mg, 0.0465 mmol) in a mixture of THF/MeOH (1:1, 2 mL). The solution was stirred at 0 °C for 30 min and then at room temperature for 4 h. Aqueous $NaHSO_3$ (5%, 0.4 mL) and aqueous citric acid (10%, 0.8 mL) were added to the solution at 0 °C. After stirring for 20 min, the mixture was extracted with $CHCl_3$ (3×10 mL). The combined $CHCl_3$ extracts were washed with brine (5 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 5:1) to afford **10b** (26.3 mg, 99%) as colorless needles. Mp 89–91 °C (MeOH); $[\alpha]_D^{28}$ −102.6 (c 0.15, $CHCl_3$); IR (film) ν_{max} 3291, 2935, 1719, 1631, 1514, 1249, 1034, 755 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, major conformer) δ 7.37–7.27 (m, 5H), 7.15 (d-like, 2H, J=8.6 Hz), 6.88 (d-like, 2H, J=8.6 Hz), 5.12 (d, 1H, J=12.4 Hz), 5.07 (d, 1H, J=12.4 Hz), 5.04 (m, 1H), 4.35 (m, 1H), 4.20 (m, 1H), 4.17 (quintet, 1H, J=7.1 Hz), 3.76 (s, 3H), 3.12 (dd, 1H, J=14.4, 3.4 Hz), 2.99 (dd, 1H, J=14.4, 11.1 Hz), 2.87 (s, 3H), 1.36 (d, 3H, J=7.3 Hz), 1.29 (d, 3H, J=7.1 Hz), 1.17 (m, 1H), 0.57 (t, 3H, J=7.3 Hz), 0.29 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD, major conformer) δ 175.7 (s), 175.1 (s), 174.2 (s), 171.2 (s), 160.4 (s), 158.0 (s), 138.2 (s), 131.5 (d, 2C), 130.8 (s), 129.5 (d, 2C), 129.0 (d, 2C), 128.9 (d), 115.4 (d, 2C), 67.6 (t), 63.7 (d), 55.8 (q), 51.9 (d), 51.6 (d), 49.9 (d), 34.4 (t), 29.9 (q), 24.8 (t), 18.8 (q), 17.6 (q), 10.9 (q); HRESIMS m/z 571.2751 ($[M+H]^+$, calcd for $C_{29}H_{39}N_4O_8$, 571.2768).

3.6.4. *Hexapeptide **12b***

A solution of **11** (13.0 mg, 0.0226 mmol) in TFA (0.8 mL) was stirred at room temperature for 2 h. TFA was removed in vacuo. The residue was treated with $CHCl_3$ (5 mL) and saturated aqueous $NaHCO_3$ (5 mL), and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue, **10b** (15.5 mg, 0.0272 mmol), and HOOBt (7.4 mg, 0.045 mmol) were dissolved in THF (1 mL), to which $EDC\cdot HCl$ (8.7 mg, 0.045 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 48 h. Saturated aqueous $NaHCO_3$ (3 mL) was added to the residue and the whole was extracted with $CHCl_3$ (3×5 mL). The combined $CHCl_3$ extracts were washed with brine (3 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was subjected to HPLC (MeCN/H_2O 60:40) to afford **12b** (17.9 mg, 77%) as an amorphous solid. $[\alpha]_D^{27}$ −190.6 (c 0.16, $CHCl_3$); IR (film) ν_{max} 3293, 2935, 1739, 1639, 1514, 1249, 1218, 1029, 752 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.76 (br m), 7.44–7.07 (m), 6.90 (dd, J=8.4, 2.5 Hz), 6.87–6.83 (m), 6.84 (d, J=8.6 Hz), 6.81 (d, J=8.6 Hz), 6.781 (d, J=8.3 Hz), 6.776 (d, J=8.3 Hz), 6.63–6.47 (m), 6.37 (br m), 5.87 (dd, J=12.1, 4.8 Hz), 5.83 (m), 5.67 (br m), 5.35 (br m), 5.31 (dd, J=11.4, 3.2 Hz), 5.21 (t, J=7.9 Hz), 5.15–4.68 (m), 4.64 (dd, J=12.2, 3.6 Hz), 4.42–4.36 (m), 4.37 (d, J=1.8 Hz), 4.13 (m), 3.96 (s), 3.931 (s), 3.926 (s), 3.76 (s), 3.75 (s), 3.74 (s), 3.63 (t, J=11.2 Hz), 3.61 (t, J=11.1 Hz), 3.32–3.14 (m), 3.26 (s), 3.18 (s), 3.03 (s), 3.01 (s), 2.98 (s), 2.98–2.81 (m), 2.96 (s), 2.88 (s), 2.86 (s), 2.77 (dd, J=11.4, 3.1 Hz), 2.71 (dd, J=11.4, 3.0 Hz), 2.550 (s), 2.547 (s), 1.68 (s), 1.39 (m), 1.35 (d, J=7.0 Hz), 1.29 (d, J=6.9 Hz), 1.25 (d, J=6.9 Hz), 1.23 (d, J=7.1 Hz), 1.17 (m), 0.88 (t, J=6.8 Hz), 0.77 (t, J=7.0 Hz), 0.52 (t, J=7.3 Hz), 0.49 (t, J=7.3 Hz), 0.43 (br m); HRESIMS m/z 1049.4634 ($[M+Na]^+$, calcd for $C_{57}H_{66}N_6O_{12}Na$, 1049.4636).

3.6.5. *RA-XX (**4**)*

Palladium (10%) on charcoal catalyst (30 mg) was added to a solution of **12b** (15.6 mg, 0.0152 mmol) in EtOH (2 mL). Then, the reaction mixture was stirred at room temperature under an atmosphere of hydrogen for 2.5 h. The catalyst was filtered off, the filtrate concentrated to dryness, and the residue was dissolved in DMF (15.2 mL). To this solution were added triethylamine (21.2 μL, 0.152 mmol) and DPPA (6.6 μL, 0.031 mmol) at 0 °C, and after stirring at room temperature for 3 days, the solvent was removed under reduced pressure. Saturated aqueous $NaHCO_3$ (10 mL) was added to the residue and the whole was extracted with $CHCl_3$ (3×10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4 and filtered, and the solvent removed in vacuo. The residue was separated by HPLC (MeOH/H_2O 40:60) to give a compound [5.8 mg, 49%, $[\alpha]_D^{26}$ −213.7 (c 0.17, $CHCl_3$)], which was shown to be identical to natural **4** by comparison of their ^{1}H and ^{13}C NMR spectra, mass spectra, and optical rotations.

3.7. *O-Methylation of RA-XXI (**5**)*

(Trimethylsilyl)diazomethane (16.9 μL of a 2.0 M solution in diethyl ether, 0.0338 mmol) was added to a stirred solution of **5** (1.3 mg, 0.0017 mmol) in MeCN/MeOH (9:1, 0.5 mL) at room temperature. After stirring at room temperature for 3 days, the mixture was concentrated in vacuo. The residue was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 20:1) to afford a compound [1.2 mg, 87%, $[\alpha]_D^{25}$ −266.3 (c 0.06, $CHCl_3$)], which was shown to be identical

to natural **4** by comparison of their ^{1}H and ^{13}C NMR spectra, mass spectra, and optical rotations.

3.8. O-Methylation of RA-XXII (*6*)

(Trimethylsilyl)diazomethane (11.7 μL of a 2.0 M solution in diethyl ether, 0.0234 mmol) was added to a stirred solution of **6** (9.2 mg, 0.012 mmol) in MeCN/MeOH (9:1, 1 mL) at room temperature. The mixture was stirred at room temperature for 10 h and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 20:1) to give a compound [8.7 mg, 93%, $[\alpha]_D^{25}$ −151.7 (*c* 0.11, $CHCl_3$)], which was shown to be identical to natural **13**[11] by comparison of their ^{1}H and ^{13}C NMR spectra, mass spectra, and optical rotations.

3.9. Assay for cytotoxic activity

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed on a 96-well plate. Murine P-388 leukemia cells (3×10^3 cells) in 100 μL of RPMI-1640 medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co. Ltd., Tokyo, Japan) and kanamycin (100 μg/mL) were placed into each well and incubated at 37 °C in a humidified atmosphere of 7% CO_2. After 24 h incubation, samples of test compounds at various concentrations (10 μL) were added to the cultures, and the mixtures were incubated for 48 h at 37 °C. Then, 20 μL of an MTT solution (5 mg/mL) was added to each well. After a further incubation for 4 h, 100 μL of 10% sodium dodecyl sulfate−0.01 M HCl solution was added to each well and the formazan crystals formed in each well were dissolved by stirring with a pipette. Optical density was recorded on a microplate reader (Tosoh MPR-A4i) at 550 nm. The cytotoxic activities in Table 3 represent the average of three replicate measurements for each test.

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