HETEROCYCLES, Vol. 65, No. 3, 2005, pp. 563 - 578
Received, 18th November, 2004, Accepted, 14th January, 2005, Published online, 21st January, 2005

ABSOLUTE STEREO-STRUCTURE OF KENDARIMIDE A, A NOVEL MDR MODULATOR, FROM A MARINE SPONGE

Naoyuki Kotoku, Liwei Cao, Shunji Aoki, and Motomasa Kobayashi*

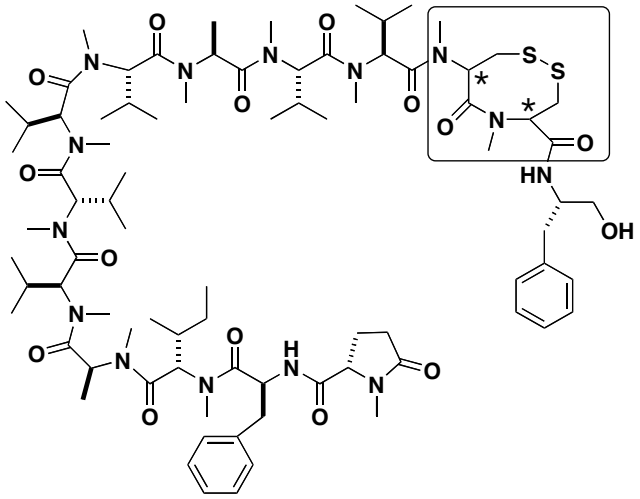
Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka 1-6, Suita, Osaka 565-0871, Japan; E-mail: kobayasi@phs.osaka-u.ac.jp.

Abstract - The absolute stereostructure of two *N*-methylcysteines in kendarimide A (**1**), a novel linear peptide reversing multidrug resistance (MDR) mediated by P-glycoprotein, was determined by comparison with synthetic model compounds. For this purpose, four diastereomers (**17a-d**) of the C-terminal tetrapeptides in kendarimide A (**1**), containing L,L-, L,D-, D,L-, D,D-*N*-methylcysteinyl-*N*-methylcysteine, were synthesized. The absolute configuration of both the two adjacent *N*-methylcysteines in **1**, which form an eight-membered disulfide ring (*ox*-[MeCys-MeCys]), was elucidated to be L-configuration by comparison of the NMR spectral data of the four model tetrapeptides with that of kendarimide A (**1**).

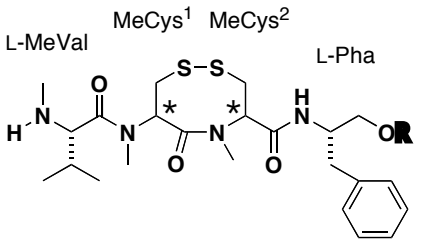
INTRODUCTION

As a part of our study of biologically active substances from marine organisms, we have been searching for new modulators of multidrug resistance (MDR) in tumor cells and have isolated kendarimide A (**1**), a novel modulator of P-glycoprotein (P-gp)-mediated MDR, from a marine sponge of *Haliclona* sp. collected at Sulawesi Island, Indonesia.[1] The chemical structure of **1** was characterized to be a linear tetradeca-peptide composed of *N*-methylpyroglutamic acid (pyroMeGlu), an eight-membered disulfide-containing *N*-methylcysteinyl-*N*-methylcysteine (*ox*-[MeCys-MeCys]), and many *N*-methylamino acid residues (Figure 1). The plane structure of **1** was elucidated by analyses of 1D, 2D-NMR spectra and the fragmentation in FAB MS spectrum. The absolute configurations of twelve amino acid residues in **1** were determined to be all L-configuration by Marfey's method except for two *N*-methylcysteines, which decomposed during acidic hydrolysis of **1**. So far, kendarimide A (**1**) was the first example of peptide which have two adjacent *N*-methylated cysteines forming an eight-membered disulfide ring (*ox*-[MeCys-MeCys]). A few examples of peptides or proteins, such as malformin A[2] and the nicotinic acetylcholine

receptor unit,[3] are known as cysteinyl-cysteine forming an eight-membered disulfide ring (*ox*-[Cys-Cys]). The eight-membered disulfide ring (*ox*-[Cys-Cys]) is known to be able to exist in different conformational states,[4,5] since it is a highly constrained structure element. A complex NMR spectrum has been observed for the *ox*-[Cys-Cys] part of the nicotinic acetylcholine receptor unit, while kendarimide A (**1**) showed a simple NMR spectrum assignable as a single conformation. Then, with the aim of determining the configurations of the two *N*-methylcysteines in **1**, four tetrapeptide diastereomers (**17a-d**) of the C-terminus, containing an eight-membered L,L-, L,D-, D,L-, D,D-*N*-methylcysteinyl-*N*-methylcysteine ring, were synthesized (Figure 1). Herein, we report full details of the synthesis of the model tetrapeptides (**17a-d**) and the determination of the absolute configuration of *N*-methylcysteines in **1** by comparison of their NMR spectral data with those of **1**.



kendarimide A (**1**)



16a: L-MeVal-L-MeCys^1-L-MeCys^2-L-Pha , R= Ac
16b: L-MeVal-L-MeCys^1-D-MeCys^2-L-Pha , R= Ac
16c: L-MeVal-D-MeCys^1-L-MeCys^2-L-Pha , R= Ac
16d: L-MeVal-D-MeCys^1-D-MeCys^2-L-Pha , R= Ac

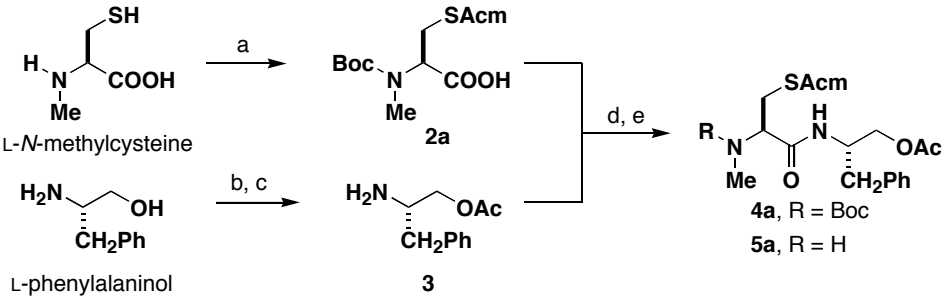
17a: L-MeVal-L-MeCys^1-L-MeCys^2-L-Pha , R= H
17b: L-MeVal-L-MeCys^1-D-MeCys^2-L-Pha , R= H
17c: L-MeVal-D-MeCys^1-L-MeCys^2-L-Pha , R= H
17d: L-MeVal-D-MeCys^1-D-MeCys^2-L-Pha , R= H

Figure 1. Chemical structures of kendarimide A and model tetrapeptides.

RESULTS AND DISCUSSION

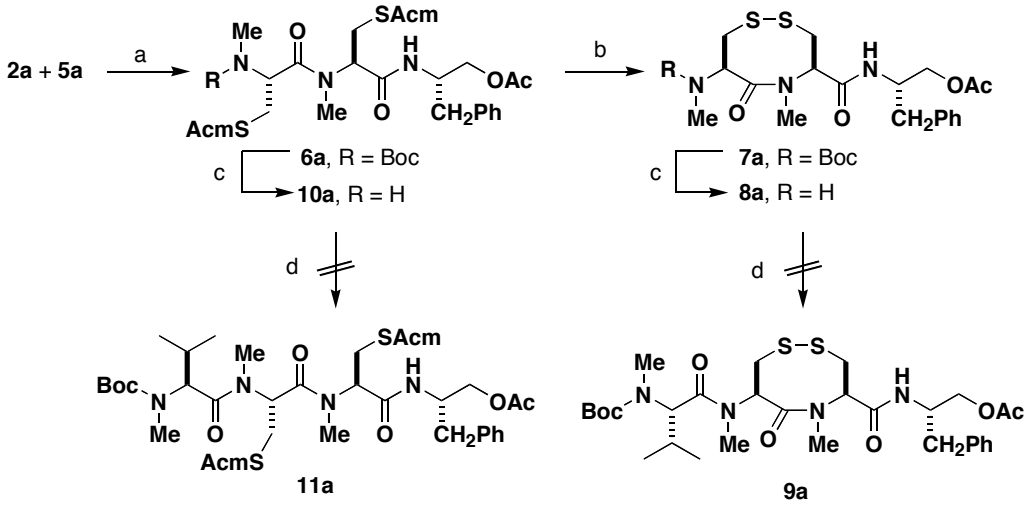
The objective tetrapeptides at the C-terminus of kendarimide A (**1**) were synthesized in order from L-phenylalaninol to L-*N*-methylvaline by one-by-one coupling. We started from the synthesis of compound (**17a**) (L-MeVal-*ox*-[L-MeCys-L-MeCys]-L-Pha). *N*-Methylcysteine could be prepared by Birch reduction of thiazolidine-4-carboxylic acid,[6] which was easily synthesized from cysteine hydrochloride and 37% formaldehyde.[7] Sequential *S*- and *N*-protections with an acetamidomethyl (Acm) group and a *tert*-butoxycarbonyl (Boc) group, respectively, gave *N*-Boc-MeCys(Acm)-OH (**2a**), an important precursor to form a disulfide-bridge of the MeCys residue (Scheme 1). Coupling of **2a** with **3**, which was obtained from L-phenylalaninol, by diethyl phosphorocyanidate (DEPC) in the presence of triethylamine (TEA) provided a dipeptide (**4a**) in high yield (96%). Following removal of the *N*-protecting Boc group gave an

amine (**5a**).



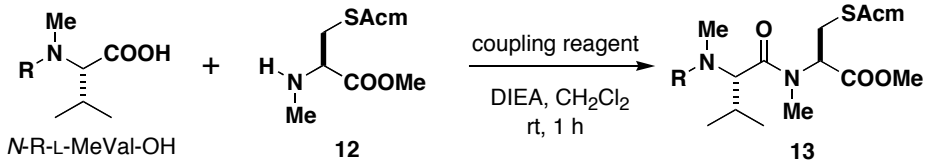
Scheme 1. *Reagents and conditions*: a) i. acetamidomethanol, conc. HCl; ii. $(Boc)_2O$, 1N NaOH, 42% in 2 steps. b) i. $(Boc)_2O$, 1N NaOH; ii. Ac_2O, pyridine, 91% in 2 steps. c) TFA, CH_2Cl_2, 97%. d) DEPC, Et_3N, DMF, 96%. e) TFA, CH_2Cl_2, quant.

Coupling reaction of **5a** with **2a** could not proceed under the above conditions or by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt). [8] Exchange of the additive from HOBt to 1-hydroxy-7-azabenzotriazole (HOAt) [9] and the solvent from CH_2Cl_2 to CH_2Cl_2-DMF (2:1) led to successful synthesis of a tripeptide (**6a**) (Scheme 2). Subsequent treatment with iodine[10] afforded compound (**7a**) through oxidative *S*-deprotection and disulfide bond formation. Boc deprotection of **7a** with TFA led to an amine (**8a**). However, condensation of **8a** with *N*-Boc-MeVal-OH could not proceed to give the desired tetrapeptide (**9a**) under the condition of EDCI/HOAt. Condensation of **10a**, a deprotected form of the tripeptide (**6a**), with *N*-Boc-MeVal-OH was also unsuccessful.



Scheme 2. *Reagents and conditions*: a) EDCI, HOAt, CH_2Cl_2/DMF, 58%. b) I_2, CH_2Cl_2/MeOH, 86%. c) TFA, CH_2Cl_2, quant. d) *N*-Boc-L-MeVal-OH, EDCI•HCl, HOAt, CH_2Cl_2/DMF.

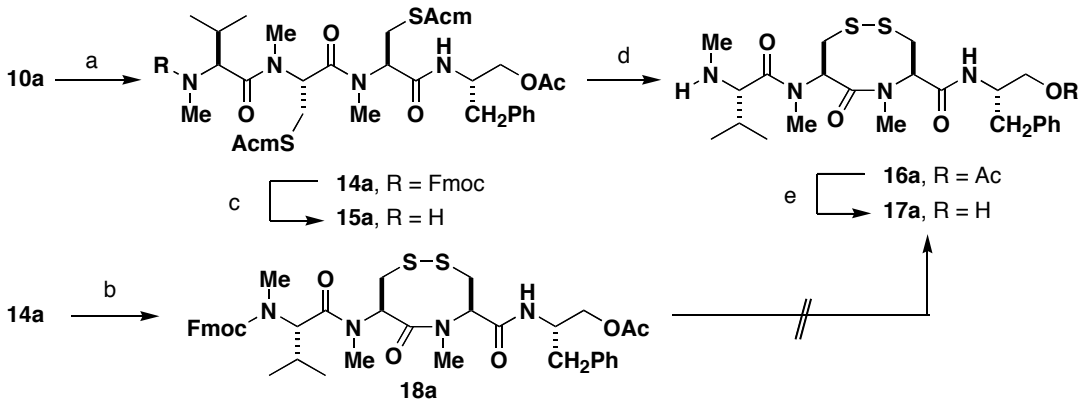
N-Methylated amino acid residues are found in many biologically active peptides, such as cyclosporins,[11] theonellapeptolides[12] and didemnins.[13] Formation of a peptide bond between *N*-methylated amino acids is difficult, and many general reagents for peptide bond-formation are ineffective. To resolve this problem and meet the demand for efficient synthesis of these sterically hindered peptides, novel coupling reagents have been developed.[14] Bis(2-oxo-3-oxazolidinyl)phosphinic chloride[15] (BOP-Cl), which was successfully employed in the synthesis of cyclosporin A, and bromotris(dimethylamino)phosphonium hexafluorophosphate[16] (Brop), which was effective even in the case of *N*-alkylated amino acids, attracted our attention. Choice of the protecting group of N-H is also important for successful coupling, and 9-fluorenylmethoxycarbonyl (Fmoc) and benzyloxycarbonyl (Cbz) groups were effective in the synthesis of cyclosporin A.[17] On the basis of this information, we carried out a model synthesis to find an effective method as shown in Scheme 3. The coupling reaction of Fmoc- or Cbz-protected *N*-methylvaline with L-MeCys(Acm)-OMe (**12**) by Brop and diisopropylethylamine (DIEA) proceeded to give the corresponding dipeptide (**13**), while the coupling reaction using BOP-Cl failed regardless of the *N*-protecting group of *N*-methylvaline.



Entry	R	Yield of **13** (%)	
		BOP-Cl	Brop
1	Boc	-	-
2	Cbz	-	34
3	Fmoc	-	30

Scheme 3.

Taking the convenience of deprotection into consideration, *N*-Fmoc-protected *N*-methylvaline was selected for the coupling unit with tripeptide (**10a**), and the desired tetrapeptide (**14a**) was obtained in 77% yield (Scheme 4). Following formation of a disulfide bond by oxidation with iodine, compound (**14a**) was readily converted to an eight-membered disulfide (**18a**). However, Fmoc deprotection of **18a** in the presence of diethylamine gave a complex mixture instead of the desired tetrapeptide (**16a**). Therefore, removal of the Fmoc group of **14a** was executed beforehand to give **15a**, and subsequent treatment with iodine led to the desired tetrapeptide acetate (**16a**). Finally, Na_2CO_3 treatment of compound (**16a**) in 70% EtOH gave the desired tetrapeptide (**17a**).



Scheme 4. *Reagents and conditions*: a) *N*-Fmoc-L-MeVal-OH, Brop, DIEA, CH_2Cl_2, 77%. b) I_2, CH_2Cl_2/MeOH, 88%. c) Et_2NH, CH_2Cl_2, 81%. d) I_2, CH_2Cl_2/MeOH, 67%. e) Na_2CO_3, 70% EtOH, 74%.

Following the procedure of the synthesis of the tetrapeptide acetate (**16a**) (*ox*-LLLL-OAc (= *ox*-L-MeVal-XX-L- Pha-OAc type; XX= L,L-*N*-methylcysteinyl-*N*-methylcysteine)) and its deacetylated analog (**17a**, *ox*-LLLL-OH) , other diastereomeric tetrapeptide acetates (**16b** (*ox*-LLDL-OAc), **16c** (*ox*-LDLL-OAc), and **16d** (*ox*-LDDL-OAc)) and their deacetylated analogs (**17b** (*ox*-LLDL-OH), **17c** (*ox*-LDLL-OH), and **17d** (*ox*-LDDL-OH)) were also prepared. All the proton and carbon signals of four acetates (**16a** – **16d**) were assigned by analysis of 1D- and 2D-NMR (COSY, HMQC, and HMBC) spectra as shown in Tables 1 and 2. Each of the tetrapeptide acetates (**16a**-**16d**) showed a similar ^{1}H-NMR spectrum except for N-H proton in phenylalaninol moiety and N-Me proton in MeCys^2 moiety. Thus, the N-H proton signals of phenylalaninol moiety for **16b** and **16c** and the N-Me proton signals of MeCys^2 for **16a** and **16d** appeared at higher fields. On the other hand, the ^{13}C-NMR spectra of those tetrapeptide acetates were classified into two groups (group A for **16a** and **16d**; group B for **16b** and **16c**). Thus, the carbon signals of C-1 and C-3 in MeCys^1 and C-2 in MeCys^2 for **16b** and **16c** were observed at lower fields. The chemical shifts of the characteristic proton and carbon signals for **16a** and **16d** resembled to those for **1**. However, the presence of an acetyl group in **16a**-**16d** made difficult to further comparison with the ^{1}H-NMR spectrum of **1**.

The deacetylated tetrapeptides (**17a**-**17d**) were unstable, in particular, compounds **17b** (*ox*-LLDL-OH) and **17c** (*ox*-LDLL-OH) were readily decomposed even when kept in a freezer. For this reason, we could compare only the respective ^{1}H-NMR spectral data for **17a**-**17d** (Table 3). Fortunately, each of the four diastereomers (**17a**-**17d**) showed the characteristic ^{1}H-NMR spectrum, respectively. The chemical shifts of some signals assignable to the L-phenylalaninol in **17b**, **17c**, and **17d** were significantly different with those of **1**. For example, the N-H proton signals in **17b** and **17c** appeared at higher field than that of **1**, and the each hydroxymethyl proton of the phenylalaninol moiety in **17c** and **17d** is overlapped and indistinguishable. The proton signals assignable to the terminal tetrapeptide in kendarimide A (**1**) were closely similar to those of **17a**. Thus, both *N*-methylcysteines in **1** were determined to be L-configuration,

Table 1. ^{1}H-NMR spectral data for the C-terminal portion of kendarimide A and model tetrapeptide acetates (*ox*-LXXL-OAc type, $CDCl_3$, 500 MHz).

C/no.	δH (*J* in Hz)				
	kendarimide A	*ox*-LLLL-OAc (**16a**)	*ox*-LLDL-OAc (**16b**)	*ox*-LDLL-OAc (**16c**)	*ox*-LDDL-OAc (**16d**)
MeVal					
2	5.28, d (11.0)	3.27#	3.26#	3.49, d-like	3.27#
3	2.27#	1.98, m	1.92, m	1.97, m	2.43
3-Me	0.96, d (6.5)	1.04, t-like	1.02, d (6.7)	1.02, d (6.7)	1.13
4	0.77, d (6.5)	1.04, t-like	0.99, d (6.7)	1.00, d (6.7)	1.11
NMe	2.96, s	2.37, s	2.34, s	2.42, s	2.54, s
MeCys[1]					
2	5.56, dd like	5.65, d-like	5.43#	5.44#	5.54, d-like
3a	3.23, dd (13.6, 11.7)	3.25#	3.60, t-like	3.35#	3.22#
3b	2.86#	2.90#	3.22, t-like	3.09#	2.98#
NMe	3.29, s	3.23, s	3.25, s	2.94, s	3.24, s
MeCys[2]					
2	5.23, dd like	5.13, dd (11.6, 4.3)	5.40#	5.42#	5.11, dd (11.6, 3.7)
3a	3.33, dd (14.2, 4.0)	3.23#	3.40#	3.59, t-like	2.96#
3b	2.85#	2.95#	3.12, dd-like	3.12, t-like	2.93#
NMe	2.34, s	2.14, s	3.27, s	3.27, s	2.68, s
Pha*					
1a	3.77, dd (11.7, 3.0)	4.25, dd (11.3, 4.6)	4.11, dd-like	4.10#	4.17, dd (11.0, 4.0)
1b	3.23, dd (11.7, 4.3)	4.15, dd (11.3, 5.8)	4.02, dd (11.3, 5.8)	4.10#	4.02, dd (11.0, 6.7)
2	4.28, m	4.45, m	4.38, m	4.43, m	4.42, m
3a	2.95#	2.95#	2.86#	2.91, dd-like	2.98#
3b	2.83#	2.74#	2.82#	2.78, dd-like	2.90#
ArH	7.20-7.26, m	7.13-7.26, m	7.16-7.31, m	7.17-7.36, m	7.23-7.28, m
NH	7.37, d (8.7)	7.40, d (8.5)	6.66, d (8.5)	6.34	7.63, d (7.9)
$COCH_3$	-	2.10, s	2.07, s	2.08, s	2.01, s

* phenylalaninol; # overlapped

and the total absolute stereostructure of kendarimide A (**1**) was clarified.

EXPERIMENTAL

The following instruments were used to obtain physical data: JEOL JMS SX-102 for FABMS spectra; JEOL JNM Lambda-500 (500 MHz) and Varian unity inova 600 (600 MHz) for ^{1}H-NMR and ^{13}C-NMR spectra. Silica gel (Fuji silysia BW-200) was used for column chromatography. Pre-coated thin layer chromatography (TLC) plates (Merck, Kieselgel, $60F_{254}$) were used for TLC. Spots on TLC plates were detected by spraying acidic *p*-anisaldehyde solution (*p*-anisaldehyde 25 ml, *c*-H_2SO_4 25 ml, acetic acid 5 ml, EtOH 425 ml) and ninhydrin solution (ninhydrin 2 g in sat. aq. n-BuOH) with subsequent heating. All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy. All the reaction solvents were distilled prior to use according to standard procedure. *N*-Boc-MeCys(Acm)-OH (**2**) was prepared by literature procedure.[8]

Abbreviations Acm; acetamidomethyl, Boc; *tert*-butoxycarbonyl, Brop; bromotris(dimethylamino)-

Table 2. ^{13}C-NMR spectral data for the C-terminal portion of kendarimide A and model tetrapeptide acetates (*ox*-LXXL-OAc type, $CDCl_3$, 150 MHz).

C/no.	δc				
	kendarimide A	*ox*-LLLL-OAc (**16a**)	*ox*-LLDL-OAc (**16b**)	*ox*-LDLL-OAc (**16c**)	*ox*-LDDL-OAc (**16d**)
MeVal					
1	172.88	174.52	175.64	172.71	172.84
2	57.78	64.93	65.54	64.48	63.81
3	27.72	23.40	31.13	30.77	30.68
3-Me	19.50	18.41	19.89	19.17	18.86
4	17.98	16.45	17.99	18.45	18.54
NMe	30.30	34.56	35.05	34.04	33.52
MeCys[1]					
1	173.00	172.95	179.43	179.00	172.84
2	56.63	55.98	53.08	53.45	57.24
3	38.80	38.70	47.23	47.10	38.51
NMe	32.50	32.04	31.49	32.20	33.06
MeCys[2]					
1	167.91	167.97	168.57	168.52	167.73
2	59.25	58.84	62.87	62.75	58.93
3	37.17	37.39[a]	38.84	38.90	37.39[b]
NMe	28.01	27.43	32.65	32.52	28.02
Pha*					
1	64.60	65.95	64.46	65.15	65.19
2	52.94	50.12	49.98	49.88	50.62
3	37.02	37.51[a]	37.55	37.32	37.45[b]
4	137.83	137.32	136.72	136.95	137.48
5/9	129.20	129.03	129.14	129.14	129.34
6/8	128.60	128.56	128.67	128.85	128.37
7	126.55	126.64	126.85	126.88	126.55
CO	-	171.00	170.78	170.95	170.81
CH_3	-	20.89	20.66	20.84	20.85

* phenylalaninol

a, b : Assignment may be interchangeable.

phosphonium hexafluorophospate, Cbz; benzyloxycarbonyl, BOP-Cl; bis(2-oxo-3-oxazolidinyl)phosphinic chloride, DIEA; diisopropylethylamine, DMF; *N*,*N*-dimethylformamide, EDCI; 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, TEA; triethylamine, EtOAc; ethyl acetate, Et_2NH; diethylamine, Fmoc; 9-fluorenylmethoxycarbonyl, HOAt; 1-hydroxy-7-azabenzotriazole, HOBt; 1-hydroxybenzotriazole, MeCys; *N*-methylcysteine, MeOH; methanol, MeVal; *N*-methylvaline, Pha; phenylalaninol.

L-Pha-OAc (3). L-Phenylalaninol (0.8 g, 5.3 mmol) was dissolved in 1 N aqueous NaOH (6.4 mL, 6.4 mmol), and di-*tert*-butyl dicarbonate (1.28 g, 5.8 mmol) was added. The reaction mixture was stirred at 25 °C for 12 h and then brought to pH 3 by adding 1 N aqueous $KHSO_4$. The resulting solution was extracted with EtOAc (3 x 30 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude *N*-Boc-L-Pha-OH. To the pyridine solution

Table 3. ^{1}H-NMR spectral data for the C-terminal portion of kendarimide A and model tetrapeptides (*ox*-LXXL-OH type, $CDCl_3$, 500 MHz).

C/no.	δH (*J* in Hz)				
	kendarimide A	*ox*-LLLL-OH (**17a**)	*ox*-LLDL-OH (**17b**)	*ox*-LDLL-OH (**17c**)	*ox*-LDDL-OH (**17d**)
MeVal					
2	5.28, d (11.0)	3.28#	3.23#	3.18#	3.26#
3	2.27#	1.95, m	1.82, m	1.91, m	1.95, m
3-Me	0.96, d (6.5)	1.03, d (6.5)	0.99, d (6.7)	1.02#	1.01, t-like
4	0.77, d (6.5)	0.99, d (6.5)	0.89, d (6.7)	1.02#	1.01, t-like
NMe	2.96, s	2.33, s	2.95, s	2.96, S	2.74, s
MeCys[1]					
2	5.56, dd like	5.71, d-like	5.33, dd (11.0, 3.1)	5.52, dd (11.0, 3.7)	5.66, d-like
3a	3.23, dd (13.6, 11.7)	3.25#	3.02#	2.98#	3.29#
3b	2.86#	2.89#	2.97#	2.65#	2.91#
NMe	3.29, s	3.24, s	2.96, s	3.23, s	3.26, s
MeCys[2]					
2	5.23, dd like	5.25, dd (11.9, 4.0)	5.29, dd (11.6, 4.9)	5.33, dd (12.2, 4.3)	5.18, dd (11.9, 4.0)
3a	3.33, dd (14.2, 4.0)	3.30#	2.83#	3.26#	3.21#
3b	2.85#	2.87#	2.78#	2.97#	3.02#
NMe	2.34, s	2.35, s	2.32, s	2.35, s	2.32, s
Pha*					
1a	3.77, dd (11.7, 3.0)	3.79, dd (11.6, 3.0)	3.58, dd (11.6, 3.7)	3.60#	3.58#
1b	3.60, dd (11.7, 4.3)	3.62, dd (11.6, 4.0)	3.43, dd (11.6, 5.5)	3.60#	3.58#
2	4.28, m	4.27, m	4.16, m	4.20, m	4.05, m
3a	2.95#	2.95#	2.95#	3.58#	3.06#
3b	2.83#	2.85#	2.84#	2.83#	2.95#
ArH	7.20-7.26, m	7.16-7.28, m	7.20-7.32, m	7.22-7.30, m	7.20-7.32, m
NH	7.37, d (8.7)	7.44, d (8.5)	6.54, d (8.5)	6.81, d (8.5)	7.83, d (7.3)

* phenylalaninol; # overlapped

(10.6 mL) of the above crude product, acetic anhydride (4.4 mL, 47 mmol) was added at 0 °C and stirred at 25 °C for 12 h. The reaction mixture was poured into ice-cold water (30 mL) and extracted with Et_2O (3 x 30 mL). The combined organic phase was washed with 0.5 N aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (25% EtOAc–*n*-hexane eluent) of the crude product afforded *N*-Boc-L-Pha-OAc (1.41 g, two steps, 91%) as a white powder. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.19-7.33 (5H, m), 4.68 (1H, m), 4.05 (2H, t-like), 2.82 (2H, dd-like), 2.11 (3H, s), 1.43 (9H, s).

To a solution of *N*-Boc-L-Pha-OAc (0.70 g, 2.4 mmol) in CH_2Cl_2 (7.0 mL), TFA (7.0 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (7.0 mL) and benzene (20 mL), and evaporated in vacuo to give a TFA salt of **3** (0.71 g, 97%) as a white amorphous solid. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.20-7.37 (5H, m), 4.32 (1H, dd, *J* = 12.5, 2.8 Hz), 4.12 (1H, dd, *J* = 12.5, 6.4 Hz), 3.70 (1H, m), 3.10 (1H, dd, *J* = 13.7, 6.4 Hz), 2.95 (1H, dd, *J* = 13.7, 8.9 Hz), 2.07 (3H, s).

***N*-Boc-L-MeCys(Acm)-L-Pha-OAc (4a).** TFA salt of **3** (61 mg, 0.20 mmol) and **2a** (64 mg, 0.21 mmol) were dissolved in DMF (0.87 mL), and DEPC (30 μL, 0.24 mmol) was added at 0 °C. After 15 min, TEA (45 μL, 0.40 mmol) was added and the reaction mixture was stirred at 0 °C for 2 h, and then at 25 °C for 12 h. The reaction solution was diluted with EtOAc/benzene (v/v, 3:1, 20 ml) and washed with 1M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (EtOAc eluent) of the crude product gave dipeptide (**4a**) (96 mg, 96%) as a white amorphous solid. FAB MS: *m/z* 482 $(M+H)^+$. HR-FAB MS: *m/z* 482.2301, calcd for $C_{23}H_{36}N_3O_6S$, Found 482.2313. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.16-7.31 (5H, m), 6.66 (1H, br s), 6.38 (1H, d-like), 4.80 (1H, t-like), 4.57 (1H, dd-like), 4.41 (1H, m), 4.10 (3H, m), 3.05 (1H, dd, *J* = 14.6, 6.1 Hz), 2.86 (1H, dd, *J* = 14.0, 6.7 Hz), 2.73-2.80 (2H, m), 2.72, 2.50 (total 3H, both br s), 2.10 (3H, s), 2.00 (3H, s), 1.49 (9H, s).

***N*-Boc-L-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (6a).** To a solution of **4a** (96 mg, 0.19 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C, TFA (1.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h and then concentrated in vacuo. The residue was dissolved in $CHCl_3$ (30 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give **5a** (69 mg, quant.) as a white amorphous solid. FAB MS: *m/z* 382 $(M+H)^+$. HR-FAB MS: *m/z* 382.1805, calcd for $C_{18}H_{28}N_3O_4S$, Found 382.1803. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.53 (1H, d, *J*= 8.5 Hz), 7.16-7.29 (5H, m), 6.69 (1H, br s), 4.39 (1H, m), 4.26 (1H, dd, *J* = 14.0, 6.7 Hz), 4.11 (1H, dd, *J* = 11.3, 4.6 Hz), 4.05 (1H, dd, *J* = 11.3, 5.8 Hz), 3.84 (1H, dd, *J* = 14.0, 5.5 Hz), 3.14 (1H, t-like), 2.88 (1H, dd, *J* =

14.0, 6.7 Hz), 2.82 (1H, dd, *J* = 14.5, 4.0 Hz), 2.78 (1H, dd, *J* = 14.0, 4.6 Hz), 2.71 (1H, dd, *J* = 14.5, 7.6 Hz), 2.30 (3H, s), 2.07 (3H, s), 1.97 (3H, s).

A solution of **2a** (61 mg, 0.20 mmol) in DMF/CH_2Cl_2 (v/v, 1:2, 1.0 mL) was treated with HOAt (270 mg, 0.40 mmol) and EDCI (380 mg, 0.40 mmol) at 0 °C for 15 min under stirring. To the reaction mixture, a DMF/CH_2Cl_2 solution (v/v, 1:2, 1.0 mL) of **5a** (74 mg, 0.19 mmol) was added, and the reaction mixture was stirred at 25 °C for additional 12 h. The reaction mixture was poured into 1 N aqueous HCl and extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (9% MeOH–EtOAc eluent) of crude products afforded **6a** (77 mg, two steps, 58 %) as a white amorphous solid. FAB MS: *m/z* 670 $(M+H)^+$. HR-FAB MS: *m/z* 670.2944, calcd for $C_{30}H_{48}N_5O_8S_2$, Found 670.2975. A mixture of multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.13-7.24 (5H, m), 6.38-7.06 (3H, d, t-like), 5.23, 5.18 (1H, dd-like), 5.06, 5.11 (1H, dd-like), 4.67 (1H, dd, *J* = 11.0, 4.9 Hz), 4.51 (1H, dd-like), 4.36 (1H, m), 3.93-4.24 (4H, m), 2.45-3.06 (12H, m), 1.94-2.03 (9H, series of s), 1.41-1.45 (9H, series of s).

***N*-Boc-*ox*-[L-MeCys-L-MeCys]-L-Pha-OAc (7a).** To a solution of iodine (55 mg, 0.216 mmol) in CH_2Cl_2-MeOH (10:1, 22 mL), **6a** (14 mg, 0.021 mmol) in CH_2Cl_2 (6.6 mL) was added dropwise, and the reaction mixture was stirred at 25 °C for 12 h. The reaction mixture was cooled in an ice bath, and 5% aqueous $Na_2S_2O_3$ was added until the color of iodine disappeared. The reaction mixture was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (50% EtOAc–*n*-hexane eluent) of the crude product afforded **7a** (9.5 mg, 86%) as a white amorphous solid. FAB MS: *m/z* 526 $(M+H)^+$. HR-FAB MS: *m/z* 526.2046, calcd for $C_{24}H_{36}N_3O_6S_2$, Found 526.2071. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.14-7.28 (5H, m), 5.33 (1H, d, *J* = 11.0 Hz), 5.10 (1H, dd, *J* = 11.6, 4.3 Hz), 4.43 (1H, m), 4.28 (1H, dd, *J* = 11.3, 4.6 Hz), 4.05 (1H, dd, *J* = 11.3, 5.2 Hz), 3.74 (1H, br s), 3.18 (2H, m), 3.03 (3H, s), 2.83-2.97 (2H, m), 2.69 (1H, dd, *J* = 13.4, 10.4 Hz), 2.20 (3H, s), 2.09 (3H, s), 1.50 (9H, s).

***N*-Fmoc-L-MeVal-L-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (14a).** To a solution of the compound (**6a**) (67 mg, 0.10 mmol) in CH_2Cl_2 (0.75 mL) at 0 °C, TFA (0.75 mL) was added dropwise. The mixture was stirred at 0 °C for 3 h, and then concentrated in vacuo. The residue was dissolved in $CHCl_3$ (20 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give **10a** as a white amorphous solid. To a solution of Fmoc-L-MeVal (35 mg, 0.10 mmol), **10a** (57 mg, 0.10 mmol), Brop (47 mg, 0.12 mmol) in CH_2Cl_2 (0.75 mL), and DIEA (47 μL, 0.24 mmol) was added. The reaction mixture was stirred at 25 °C for 12 h, and then CH_2Cl_2 was

evaporated. The resulting residue was dissolved in EtOAc (30 mL) and washed with 5% aqueous $KHSO_4$, brine, 5% aqueous $NaHCO_3$, brine, dried over Na_2SO_4, concentrated in vacuo. Flash chromatography (9% MeOH-EtOAc eluent) of the crude product afforded **14a** (69 mg, 77%) as a white amorphous solid. FAB MS: *m/z* 905 $(M+H)^+$. HR-FAB MS: *m/z* 905.3941, calcd for $C_{46}H_{61}N_6O_9S_2$, Found 905.3921. A mixture (3:1) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.09-7.78 (14H, m), 6.41-6.90 (2H, d-like), 5.66, 5.69 (1H, both dd, *J* = 9.8, 4.3 Hz), 5.22, 5.28 (1H, both dd, *J* = 11.0, 4.9 Hz), 3.91-4.83 (11H, series of dd or d), 2.35-3.21 (16H, series of dd or s), 1.99-2.09 (9H, series of s), 0.19-0.49, 0.81-0.86 (6H, series of d).

***N*-Fmoc-L-MeVal-*ox*-[L-MeCys-L-MeCys]-L-Pha-OAc (18a).** Synthesis of **18a** (88% yield) was executed by the method described in the synthesis of **7a**. FAB MS: *m/z* 761 $(M+H)^+$. HR-FAB MS: *m/z* 761.3043, calcd for $C_{40}H_{49}N_4O_7S_2$, Found 761.2987. A mixture (1:1) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.79 (2H, m), 7.54-7.60 (2H, m), 7.01-7.43 (10H, m), 5.28, 5.50 (1H, both d, *J* = 11.0 Hz), 5.00, 5.12 (1H, both dd-like), 4.73, 4.94 (1H, both dd, *J* = 10.9, 3.7 Hz), 3.78, 4.85 (1H, both d, *J* = 10.7 Hz), 4.38, 4.56 (1H, both dd, *J* = 10.6, 6.7 Hz), 4.32-4.45 (1H, m), 4.12-4.24 (2H, series of dd), 4.04-4.18 (1H, both dd, *J* = 11.2, 4.9 Hz), 2.57-3.23 (6H, series of dd), 2.03, 2.05, 2.09, 2.12, 2.24, 2.72, 2.88, 3.27 (12H, series of s), 2.17, 2.30 (1H, m), 0.25, 0.53, 0.88, 0.99 (6H, series of d, *J* = 6.7 Hz).

L-MeVal-L-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (15a). To a solution of **14a** (63 mg, 0.070 mmol) in CH_2Cl_2 (0.7 mL), Et_2NH (0.1 mL, 0.97 mmol) was added and stirred at 25 °C for 12 h. Reaction mixture was concentrated in vacuo, and the resulting residue was purified by flash chromatography (25% MeOH-EtOAc eluent) to provide **15a** (39 mg, 81%) as a colorless amorphous. FAB MS: *m/z* 683 $(M+H)^+$. HR-FAB MS: *m/z* 683.3261, calcd for $C_{31}H_{51}N_6O_7S_2$, Found 683.3245. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.13-7.31 (5H, m), 6.86 (2H, m), 6.60 (1H, t-like), 5.83 (1H, dd, *J* = 9.2, 4.9 Hz), 5.29 (1H, dd, *J* = 11.0, 4.9 Hz), 4.80 (1H, dd, *J* = 14.0, 7.3 Hz), 4.59 (1H, dd, *J* = 14.0, 7.3 Hz), 4.45 (1H, m), 4.05-4.16 (4H, m), 3.35 (1H, d-like), 2.68-3.14 (6H, m), 2.92 (3H, s), 2.60 (3H, s), 2.41 (3H, s), 2.09 (3H, s), 2.07 (3H, s), 2.00 (3H, s), 1.05 (3H, d, *J* = 7.0 Hz), 0.91 (3H, d, *J* = 7.0 Hz).

L-MeVal-*ox*-[L-MeCys-L-MeCys]-L-Pha-OAc (16a). Synthesis of **16a** (67% yield) was executed by the method described in the synthesis of **7a**. FAB MS: *m/z* 539 $(M+H)^+$. HR-FAB MS: *m/z* 539.2362, calcd for $C_{25}H_{39}N_4O_5S_2$, Found 539.2368. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data in $CDCl_3$ are given in Table 1 and Table 2.

L-MeVal-*ox*-[L-MeCys-L-MeCys]-L-Pha-OH (17a). To a solution of **16a** (4.6 mg, 8.6 μmol) in EtOH-H_2O (v/v, 7:3, 250 μL) Na_2CO_3 (2.9 mg, 27 μmol) was added. The reaction mixture was stirred at 25 °C for

50 min and then neutralized with 1N aqueous HCl. The resulting solution was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated, and the resulting residue was purified by flash chromatography (30% MeOH-AcOEt eluent) to give **17a** (3.1 mg, 74%) as a colorless powder. FAB MS: *m/z* 497 $(M+H)^+$. HR-FAB MS: *m/z* 497.2256, calcd for $C_{23}H_{37}N_4O_4S_2$, Found 497.2242. ^{1}H-NMR (500 MHz) data in $CDCl_3$ are given in Table 3.

***N*-Boc-D-MeCys(Acm)-L-Pha-OAc (4b).** Colorless powder. FAB MS: *m/z* 482 $(M+H)^+$. HR-FAB MS: *m/z* 482.2301, calcd for $C_{23}H_{36}N_3O_6S$, Found 482.2329. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.17-7.31 (5H, m), 6.35-6.71 (2H, br s or d-like), 4.40-4.82 (3H, t-like, dd-like or m), 4.02-4.12 (3H, m), 3.03 (1H, dd, *J* = 14.6, 6.1 Hz), 2.71-2.89 (6H, m or s), 2.07 (3H, s), 1.99 (3H, s), 1.47 (9H, s).

D-MeCys(Acm)-L-Pha-OAc (5b). Colorless powder. FAB MS: *m/z* 382 $(M+H)^+$. HR-FAB MS: *m/z* 382.1805, calcd for $C_{18}H_{28}N_3O_4S$, Found 382.1817. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.56 (1H, d, *J* = 8.5 Hz), 7.19-7.31 (5H, m), 6.81 (1H, t-like), 4.42 (1H, m), 4.37 (1H, dd, *J* = 14.0, 6.7 Hz), 4.21 (1H, dd, *J* = 14.0, 6.1 Hz), 4.14 (1H, dd, *J* = 11.0, 4.3 Hz), 4.06 (1H, dd, *J* = 11.0, 5.8 Hz), 3.16 (1H, dd, *J* = 6.7, 4.3 Hz), 2.78-2.94 (4H, series of dd), 2.30 (3H, s), 2.08 (3H, s), 2.01 (3H, s).

***N*-Boc-L-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (6b).** White amorphous solid. FAB MS: *m/z* 670 $(M+H)^+$. HR-FAB MS: *m/z* 670.2944, calcd for $C_{30}H_{48}N_5O_8S_2$, Found 670.2938. A mixture of multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 6.54-6.95, 7.91 (2H, series of d-like or t-like), 7.09-7.24 (6H, m), 3.72-4.45, 4.73, 4.99, 5.11, 5.64, 6.29 (9H, series of dd or m), 2.42-3.06 (12H, series of dd or s), 1.86-2.00 (9H, series of s), 1.35-1.43 (9H, series of s).

L-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (10b). White amorphous solid. FAB MS: *m/z* 570 $(M+H)^+$. HR-FAB MS: *m/z* 570.2420, calcd for $C_{25}H_{40}N_5O_6S_2$, Found 570.2417. A mixture (5:4) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.68 (1H, t-like), 7.16-7.28 (5H, m), 6.85 (1H, t-like), 6.78 (1H, d, *J* = 9.2 Hz), 5.28 (1H, dd, *J* = 11.0, 4.9 Hz), 4.49, 4.42 (2H, both dd, *J* = 14.0, 6.7 Hz), 4.34 (1H, m), 4.17 (1H, dd, *J* = 14.0, 5.8 Hz), 4.11 (1H, dd, *J* = 14.0, 4.6 Hz), 4.06 (1H, dd, *J* = 11.6, 4.3 Hz), 3.98 (1H, dd, *J* = 11.6, 5.5 Hz), 3.64 (1H, dd, *J* = 7.3, 4.9 Hz), 2.98 (3H, s), 2.62-2.95 (6H, series of dd), 2.34 (3H, s), 2.04 (3H, s), 1.99 (3H, s), 1.97 (3H, s).

***N*-Fmoc-L-MeVal-L-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (14b).** White amorphous solid. FAB MS: *m/z* 905 $(M+H)^+$. HR-FAB MS: *m/z* 905.3941, calcd for $C_{46}H_{61}N_6O_9S_2$, Found 905.3949. A mixture of

multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.18-7.75 (14H, m), 8.10, 8.04, 6.81-7.11 (2H, d-like or t-like), 5.18-6.58, 3.68-3.96 (13H, series of dd or m), 2.30, 2.44-3.06 (16H, series of dd, s or m), 1.86-2.09 (9H, series of s), 0.21-0.26, 0.46-0.53, 0.80-0.95 (6H, series of d, J = 6.7 Hz).

L-MeVal-L-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (15b). White amorphous solid. FAB MS: m/z 683 $(M+H)^+$. HR-FAB MS: m/z 683.3261, calcd for $C_{31}H_{51}N_6O_7S_2$, Found 683.3258. A mixture (7:3) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 8.22 (1H, d, J = 9.2 Hz), 6.92-7.69 (total 8H, series of m), 3.88-4.97, 3.30, 4.77-5.55, 6.22 (9H, dd-like, t-like or m), 2.36, 2.53-3.14 (16H, series of dd or s), 1.92-2.9 (9H, series of s), 1.88 (1H, m), 0.88-0.94, 0.97-1.03 (6H, series of d, J = 6.7 Hz).

L-MeVal-*ox*-[L-MeCys-D-MeCys]-L-Pha-OAc (16b). Colorless powder. FAB MS: m/z 539 $(M+H)^+$. HR-FAB MS: m/z 539.2362, calcd for $C_{25}H_{39}N_4O_5S_2$, Found 539.2364. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data in $CDCl_3$ are given in Table 1 and Table 2.

L-MeVal-*ox*-[L-MeCys-D-MeCys]-L-Pha-OH (17b). Colorless powder. FAB MS: m/z 497 $(M+H)^+$. HR-FAB MS: m/z 497.2256, calcd for $C_{23}H_{37}N_4O_4S_2$, Found 497.2230. ^{1}H-NMR (500 MHz) data in $CDCl_3$ are given in Table 3.

***N*-Boc-D-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (6c).** White amorphous solid. FAB MS: m/z 670 $(M+H)^+$. HR-FAB MS: m/z 670.2944, calcd for $C_{30}H_{48}N_5O_8S_2$, Found 670.2933. A mixture of multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.02-7.23 (6H, m), 6.24, 6.53-6.85, 8.02 (2H, series of d or t-like), 3.72-5.15, 5.52, 5.70 (9H, series of dd or m), 2.29-3.06 (12H, series of dd or s), 1.88-2.01 (9H, series of s), 1.33-1.44 (9H, series of s).

D-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (10c). White amorphous solid. FAB MS: m/z 570 $(M+H)^+$. HR-FAB MS: m/z 570.2420, calcd for $C_{25}H_{40}N_5O_6S_2$, Found 570.2408. A mixture (5:4) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.73 (1H, t-like), 7.16-7.28 (5H, m), 6.90 (1H, t-like), 6.70 (1H, d, J = 8.5 Hz), 5.31, (1H, dd, J = 10.4, 4.9 Hz), 4.50, 4.53 (2H, both dd, J = 7.0, 4.6 Hz), 4.39 (1H, m), 4.02-4.17 (4H, series of dd), 3.59 (1H, dd, J = 7.3, 4.3 Hz), 3.06 (1H, dd, J = 15.0, 5.2 Hz), 2.63-2.94 (8H, series of dd or s), 2.36 (3H, s), 2.09 (3H, s), 2.00 (3H, s), 1.99 (3H, s).

***N*-Fmoc-L-MeVal-D-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (14c).** White amorphous solid. FAB MS: m/z 905 $(M+H)^+$. HR-FAB MS: m/z 905.3941, calcd for $C_{46}H_{61}N_6O_9S_2$, Found 905.3957. A mixture of

multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.10-7.75 (14H, m), 8.29, 6.46-6.84 (2H, d-like or t-like), 5.01-6.16, 3.64-4.90 (13H, series of dd or m), 2.15-3.07 (16H, series of dd, s or m), 1.93-2.10 (9H, series of s), 0.14, 0.21, 0.51, 0.55, 0.81, 0.94 (6H, series of d, J = 6.7 Hz).

L-MeVal-D-MeCys(Acm)-L-MeCys(Acm)-L-Pha-OAc (15c). White amorphous solid. FAB MS: *m/z* 683 $(M+H)^+$. HR-FAB MS: *m/z* 683.3261, calcd for $C_{31}H_{51}N_6O_7S_2$, Found 683.3279. A mixture (1:1) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 8.22, 6.91 (1H, both d, J = 8.5 Hz), 7.10-7.29 (5.5H, m), 6.59, 6.83, 7.67 (1.5H, series of t-like), 4.83, 4.97, 5.27, 5.54, 6.24 (2.5H, dd-like or t-like), 3.81-4.61 (6.5H, series of dd or m), 2.29-3.35 (7H, series of dd or d), 2.29, 2.36, 2.51, 2.83, 2.91, 2.99 (9H, series of s), 1.98, 2.03, 2.07, 2.08 (9H, series of s), 1.90 (1H, m), 0.88, 0.93, 1.04 (6H, series of d, J = 6.7 Hz).

L-MeVal-*ox*-[D-MeCys-L-MeCys]-L-Pha-OAc (16c). Colorless powder. FAB MS: *m/z* 539 $(M+H)^+$. HR-FAB MS: *m/z* 539.2362, calcd for $C_{25}H_{39}N_4O_5S_2$, Found 539.2373. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data in $CDCl_3$ are given in Table 1 and Table 2.

L-MeVal-*ox*-[D-MeCys-L-MeCys]-L-Pha-OH (17c). Colorless powder. FAB MS: *m/z* 497 $(M+H)^+$. HR-FAB MS: *m/z* 497.2256, calcd for $C_{23}H_{37}N_4O_4S_2$, Found 497.2216. ^{1}H-NMR (500 MHz) data in $CDCl_3$ are given in Table 3.

***N*-Boc-D-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (6d).** White amorphous solid. FAB MS: *m/z* 670 $(M+H)^+$. HR-FAB MS: *m/z* 670.2944, calcd for $C_{30}H_{48}N_5O_8S_2$, Found 670.2968. A mixture of multiple conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.13-7.23 (5H, m), 6.56-7.07 (2H, d-like or t-like), 5.08-5.59 (2H, d-like or m), 3.98-4.74 (5H, m), 2.62-3.04 (12H, series of dd or s), 1.89-2.08 (9H, series of s), 1.38-1.42 (9H, series of s).

D-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (10d). White amorphous solid. FAB MS: *m/z* 570 $(M+H)^+$. HR-FAB MS: *m/z* 570.2420, calcd for $C_{25}H_{40}N_5O_6S_2$, Found 570.2431. A mixture (5:4) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 8.50, 6.91 (1H, both d, J = 8.5 Hz), 7.50-7.11 (1H, both t-like), 7.17-7.31 (5H, m), 6.72 (1H, m), 5.37, (0.5H, dd, J = 10.4, 4.9 Hz), 3.92-4.68 (8H, series of dd or m), 3.73 (0.5H, t, J = 6.1 Hz), 2.68-3.07 (6H, series of dd), 2.78, 2.96 (3H, 2s), 2.20, 2.40 (3H, both s), 1.99-2.07 (9H, series of s).

***N*-Fmoc-L-MeVal-D-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (14d).** White amorphous solid. FAB MS:

m/z 905 $(M+H)^+$. HR-FAB MS: *m/z* 905.3941, calcd for $C_{46}H_{61}N_6O_9S_2$, Found 905.3929. A mixture (3:2) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.17-7.78 (13H, m), 6.26, 6.86, 6.99, 7.05 (2H, t-like), 6.63, 6.81 (1H, both d, *J* = 8.5 Hz), 5.55, 5.76 (1H, both dd, *J* = 10.4, 4.3 Hz), 5.25, 5.10 (1H, both dd, *J* = 11.5, 4.9 Hz), 4.95 (0.6H, dd, *J* = 10.7, 3.4 Hz), 3.91-4.78 (10.4H, series of dd or m), 2.51-3.07 (6H, series of dd), 2.61-3.02 (7.5H, series of s), 2.30, 1.93 (1H, m), 1.92-2.08 (10.5H, series of s), 0.12-0.97 (6H, series of d, *J* = 6.7 Hz).

L-MeVal-D-MeCys(Acm)-D-MeCys(Acm)-L-Pha-OAc (15d). White amorphous solid. FAB MS: *m/z* 683 $(M+H)^+$. HR-FAB MS: *m/z* 683.3261, calcd for $C_{31}H_{51}N_6O_7S_2$, Found 683.3248. A mixture (4:1) of two conformers. ^{1}H-NMR (500 MHz, $CDCl_3$, □): 7.59, 6.94 (1H, both d, *J* = 8.5 Hz), 7.18-7.30 (5H, m), 7.03, 7.39 (1H, both t-like), 6.73, 6.82 (1H, both t-like), 5.88, 5.69 (1H, both dd, *J* = 9.8, 4.9 Hz), 5.33, 4.23 (1H, both dd, *J* = 11.0, 4.9 Hz), 4.66, 4.74 (1H, both dd, *J* = 14.0, 7.3 Hz), 4.53, 4.62 (1H, both dd, *J* = 14.0, 7.0 Hz), 4.36 (1H, m), 3.97-4.13 (4H, series of dd), 3.29 (1H, d-like), 2.62-3.08 (6H, series of dd), 2.86-3.00 (6H, series of s), 2.27, 2.28 (3H, both s), 1.98, 1.99, 2.00, 2.04, 2.08 (9H, series of s), 1.93 (1H, m), 0.90, 0.92, 1.01, 1.06 (6H, series of d, *J* = 6.7 Hz).

L-MeVal-*ox*-[D-MeCys-D-MeCys]-L-Pha-OAc (16d). Colorless powder. FAB MS: *m/z* 539 $(M+H)^+$. HR-FAB MS: *m/z* 539.2362, calcd for $C_{25}H_{39}N_4O_5S_2$, Found 539.2358. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data in $CDCl_3$ are given in Table 1 and Table 2.

L-MeVal-*ox*-[D-MeCys-D-MeCys]-L-Pha-OH (17d). Colorless powder. FAB MS: *m/z* 497 $(M+H)^+$. HR-FAB MS: *m/z* 497.2256, calcd for $C_{23}H_{37}N_4O_4S_2$, Found 497.2260. ^{1}H-NMR (500 MHz) data in $CDCl_3$ are given in Table 3.

ACKNOWLEDGEMENTS

The authors are grateful to the Takeda Science Foundation, the Tokyo Biochemical Research Foundation, and the Ministry of Education, Culture, Sports, Science, and Technology of Japan for financial supports.

REFERENCES

1. S. Aoki, L. Cao, K. Matsui, R. Rachmat, S. Akiyama, and M. Kobayashi, *Tetrahedron*, 2004, **60**, 7053.
2. M. Bodanszky and G. L. Stahl, *Proc. Nat. Acad. Sci. U.S.A.,* 1974, **71**, 2791.
3. N. Le Novére, P.-J. Corringer, and J.-P. Changeux, *Biophys. J.*, 1999, **76**, 2329.
4. C. J. Creighton, C. H. Reynolds, D. H. S. Lee, G. C. Leo, and A. B. Reitz, *J. Am. Chem. Soc.*, 2001, **123**, 12664.

5. D. Z. Avizonis, S. Farr-Jones, P. A. Kosen, and V. J. Basus, *J. Am. Chem. Soc.*, 1996, **118**, 13031.

6. S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, 1937, **59**, 200.

7. P. Blondeau, C. Berse, and D. Gravel, *Can. J. Chem.,* 1967, **45**, 49.

8. D. L. Boger, S. Ichikawa, W. C. Tse, M. P. Hedrick, and Q. Jin, *J. Am. Chem. Soc.*, 2001, **123**, 561.

9. L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397.

10. B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, and W. Rittel, *Helv. Chim. Acta*, 1980, **63**, 899.

11. J. F. Borel, 'cyclosporine A,' ed. by D. J. G. White, Elsevier Biomedical: Amsterdam, 1982, pp. 5-17.

12. M. Doi, T. Ishida, M. Kobayashi, J. R. Deschamps, and J. L. Flippen-Anderson, *Acta Cryst.*, 1999, **C55**, 796.

13. A. Boulanger, E. Abou-Mansour, A. Badre, B. Banaigs, B. Combaut, and C. Francisco, *Tetrahedron Lett.*, 1994, **35**, 4345.

14. a) R. D. Tung and D. H. Rich, *J. Am. Chem. Soc.*, 1985, **107**, 4342. b) E. Frérot, J. Coste, A. Pantalom, M.-N. Dufour, and P. Joum, *Tetrahedron*, 1991, **47**, 259. c) P. Li and J. C. Xu, *Tetrahedron*, 2000, **56**, 9949.

15. W. J. Colucci, R. D. Tung, J. A. Petri, and D. H. Rich, *J. Org. Chem.*, 1990, **55**, 2895.

16. J. Coste, M.-N. Dufour, A. Pantaloni, and B. Castro, *Tetrahedron Lett.*, 1990, **31**, 669.

17. R. D. Tung, M. K. Dhaon, and D. H. Rich, *J. Org. Chem.*, 1986, **51**, 3350.