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LEPTOSINS O–S, CYTOTOXIC METABOLITES OF A STRAIN OF *LEPTOSPHAERIA* SP. ISOLATED FROM A MARINE ALGA

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Abstract – Leptosins O (**1**), P (**2**), Q (**3**), R (**4**) and S (**5**) have been isolated from a strain of *Leptosphaeria* sp. originally separated from the marine alga *Sargassum tortile*. Their absolute stereostructures have been elucidated on the basis of spectroscopic analyses of their acetate derivatives (**6**–**10**) using various 1D and 2D NMR techniques and some chemical transformations. The NMR and NOE spectral analyses of **6**–**10** revealed that they exist in a single conformer of B type in $CDCl_3$. Among these metabolites, leptosins O (**1**) and P (**2**) exhibited significant cytotoxicity against cultured P388 cells

INTRODUCTION

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have chosen to seek new antitumor metabolites from microorganisms inhabiting the marine environment.[1–4] As part of this program, we reported that antitumor and cytotoxic compounds, leptosins A–N_1, belonging to a series of epipolysulfanyldioxopiperazines, were produced by a strain of *Leptosphaeria* sp. OUPS-N80 isolated from the marine alga *Sargassum tortile* C. AGAROH (Sargassaceae).[4-8] Our continuing search for cytotoxic metabolites from this fungal strain led to the isolation of five new epipoly-sulfanyldioxopiperazine designated leptosins O (**1**), P (**2**), Q (**3**), R (**4**) and S (**5**) (Figure 1). Some of these metabolites exhibited significant cytotoxic activity against the murine P388 lymphocytic leukemia cell line. We report herein their absolute stereostructures,

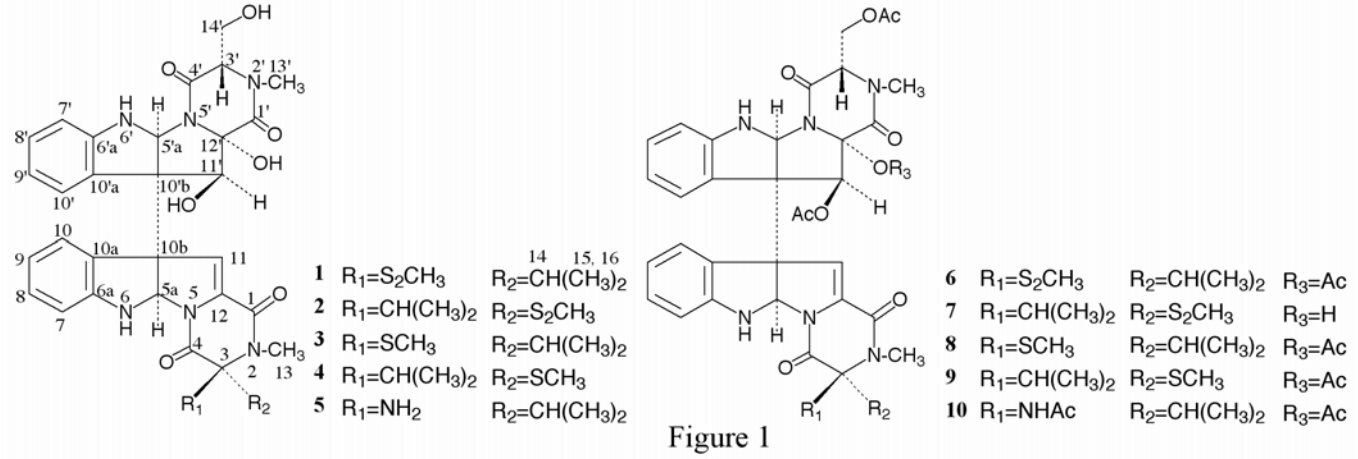


Figure 1

conformation and biological activities.

RESULTS AND DISCUSSION

The fungal strain was cultured at 27˚C for 3 weeks in a liquid medium (90 L) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5 as reported previouly.[5] The MeOH extract of the mycelium was purified by bioassay-directed fractionation (cytotoxicities against P388 cell line) employing a combination of Sephadex LH-20 and silica gel column chromatography and reversed phase HPLC to afford leptosins O–S (**1**–**5**).The stereostructures of these compounds (**1**–**5**) were examined by spectroscopic analyses using 1D and 2D NMR techniques. Their ^{1}H and ^{13}C NMR signals were so broad that it was impossible to analyze them. Therefore, the NMR spectral data of the acetates (**6–10**) derived from **1**–**5** were used for the structure analysis of **1**–**5**.

Leptosin O (**1**) is a pale yellow powder with the molecular formula $C_{33}H_{36}N_6O_7S_2$ as established by the $[M + H]^+$ peak of **1** in high-resolution secondary-ion mass spectrometry (HRSIMS). Its IR spectrum contained absorption bands at 3524, 1688, 1658, 1610 and 1599 cm^{-1}, characteristic of hydroxy, amino and amido groups, and an aromatic ring. A close inspection of the NMR spectra of the triacetate derivative (**6**) of **1** (Table 1) by DEPT and ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments and comparison with spectral data for the other leptosins[4–8] revealed the presence of the following functionalities in **6**: two methines (C-5a and C-5'a) each bearing two nitrogen atoms, one methine (C-3') bearing a nitrogen atom and a carbonyl group, one quaternary sp^3-carbon (C-3) bearing both nitrogen and sulfur atoms, one quaternary sp^3-carbon (C-12') bearing both nitrogen and oxygen atoms, four amide carbonyls (C-1, C-4, C-1' and C-4'), two *N*-methyls (C-13 and C-13'), one isopropyl (C-14, C-15 and C-16), two 1, 2-disubstituted benzenes (C-6a to 10a and C-6'a to C-10'a), each bonding to an amino group as one substituent, two benzylic quaternary sp^3-carbons (10b and 10'b), one trisubstituted double bond (C-11 and C-12), one methyldithio group (δ_H 1.97; δ_C 22.57), one acetoxymethine (C-11'), one acetoxymethyl (C-14') and another acetyl group. The connection of these functional groups except for the methyldithio and one acetyl group was determined on the basis of ^{1}H-^{1}H COSY and HMBC correlations summarized in Figure 2. The connection of the methyldithio and one acetyl groups to C-3 and C-12', respectively, was presumed by the ^{13}C chemical shift of C-3 (δ_C 83.36) and C-12' (δ_C 89.33), and 12'-OAc).[4] The presence of the acetyl group at C-12' was supported by NOE between H-5'a and acetyl protons. The relative configuration of **6** was deduced from detailed analysis of NOE data of **6** in $CDCl_3$ (Table 1 and Figure 3). NOE correlations from H-5'a to H-5a, H-15, H-14' and 12'-OAc observed in the

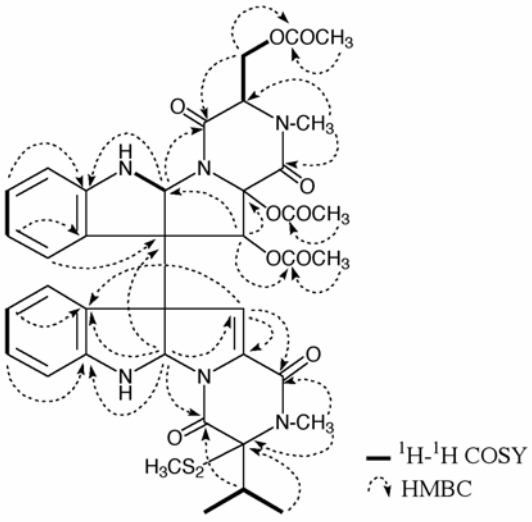


Figure 2 Selected ^{1}H-^{1}H COSY and HMBC correlations in triacetate **6** of leptosin O (**1**)

Table 1 NMR spectral data of triacetate (**6**) of leptosin O (**1**) in $CDCl_3$

Position	δ_H[a]	*J* /Hz	^{1}H-^{1}H COSY	NOEs	δ_C	HMBC (C)[c]
1					157.05 (q)[b]	
3					83.36 (q)	
4					161.93 (q)	
5 a	5.30 s			6, 5'a	79.16 (t)	4, 6a, 10a, 10b, 11, 10'b
6	5.69 s			5a		10a, 10b
6 a					148.50 (q)	
7	6.64 d	7.8 (8)	8	8	109.75 (t)	9, 10a
8	7.10 t	7.8 (7, 9)	7, 9	7, 9	129.37 (t)	6a, 10
9	6.49 t	7.8 (8, 10)	8, 10	8, 10	118.63 (t)	7, 10a
10	7.62 d	7.8 (9)	9	9, 11'	126.92 (t)	10a
10 a					121.83 (q)	
10 b					65.25 (q)	
11	6.66 s			5'a, 11', 12'-OCOC<u>H</u>$_3$	117.49 (t)	1, 10a, 12
12					133.08 (q)	
13	3.02 s			14, 15, 16	29.03 (p)	1, 3
14	2.55 heptet	6.7 (15, 16)	15, 16	13, 15, 16	35.44 (t)	3, 4, 15, 16
15	1.12 d	6.7 (14)	14	13, 14, 16, 5'a, 12'-OCOC<u>H</u>$_3$	18.22 (p)	3, 14, 16
16	1.33 d	6.7 (14)	14	13, 14, 15	17.99 (p)	3, 14, 15
3-S_2CH_3	1.97 s				22.57 (p)	
1'					160.18 (q)	
3'	4.23 dd	5.8 (14'A), 3.2 (14B')	14'A, 14'B	13', 14'A, 14'B	63.90 (t)	
4'					165.49 (q)	
5' a	6.04 s		6'	5a, 11, 15, 14'B, 12'-OCOC<u>H</u>$_3$	79.45 (t)	4', 6'a
6'	5.37 br s		5'a			6'a, 10'a
6' a					148.98 (q)	
7'	6.42 d	7.8 (8')	8'	8'	109.47 (t)	9', 10'a
8'	7.06 t	7.8 (7', 9')	7', 9'	7', 9'	129.60 (t)	6'a, 10'
9'	6.77 t	7.8 (8', 10')	8', 10'	8'	119.46 (t)	10'a
10'	5.97 d	7.8 (9')	9'		125.23 (t)	6'a, 10'b
10' a					126.78 (q)	
10' b					64.73 (q)	
11'	6.50 s			10, 11	78.04 (t)	5'a, 12', 11'-O<u>C</u>OCH$_3$
12'					89.33 (q)	
13'	2.96 s			3', 14'A, 11'-OCOC<u>H</u>$_3$	32.49 (p)	1', 3'
14' A	4.59 dd	12.2 (14'B), 5.8 (3')	3'	3', 13', 14'B	65.60 (s)	3', 4', 14'-O<u>C</u>OCH$_3$
B	4.84 dd	12.2 (14'A), 3.2 (3')	3'	3', 5'a, 14'A		3', 4', 14'-O<u>C</u>OCH$_3$
11'-OCOC<u>H</u>$_3$	1.76 s			13'	20.42 (p)	11'-O<u>C</u>OCH$_3$
11'-O<u>C</u>OCH$_3$					167.37 (q)	
12'-OCOC<u>H</u>$_3$	2.29 s			11, 15, 5'a	21.03 (p)	12'-O<u>C</u>OCH$_3$
12'-O<u>C</u>OCH$_3$					170.26 (q)	
14'-OCOC<u>H</u>$_3$	2.10 s				20.71 (p)	14'-O<u>C</u>OCH$_3$
14'-O<u>C</u>OCH$_3$					169.98 (q)	

a ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* /Hz). Figures in parentheses indicate the proton coupling with that position. *b* Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT. *c* Long range ^{1}H-^{13}C correlations from H to C observed in the HMBC experiment.

NOESY of **6** implied that H-5a, H-5'a, 3-isopropyl, 3'-acetoxymethyl groups and 12'-OAc are oriented on the same side, and H-5a and H-5'a are both *cis* to the C-10b–C-10'b bond (Figure 3). This observation

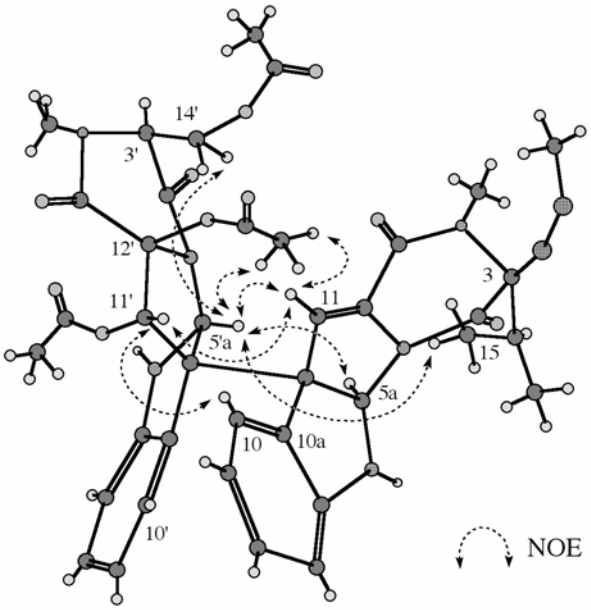
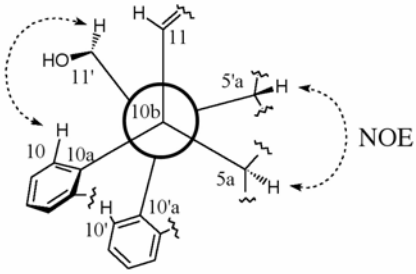


Figure 3 Observed NOEs for the triacetate derivative (**6**) of leptosin O (**1**)



Conformer B

Figure 4 Conformations of the acetate derivatives of leptosins O–S (**1**–**5**) in $CDCl_3$

also demonstrated that the isopropyl-bearing and acetoxymethyl-dioxopiperazine rings exist in chair and boat conformations, respectively. In addition, NOEs from H-11' to H-10, and H-11 to H-11' and 12'-OAc indicated that H-11' is oriented *cis* to 12'-OAc and consequently H-5'a. The relative configuration of **6** and, consequently, **1** was thus established. Previously we found that leptosins exist in either or a mixture of two conformers of A and B types, which are formed by rotation of two monomeric subunits about the C-10b–C-10'b bond.[4, 8] These conformers can be deduced from a combination of chemical shifts of H-10 and H-10', and NOE data between H-5a, H-10, H-11, H-5', H-10' and H-11'. In compound (**6**), NOEs were observed between H-10 and H-11', and H-5a and H-5'a as mentioned above, and the signals for H-10 and H-10' appeared at lower (δ 7.62) and higher (δ 5.97) fields, respectively. This evidence demonstrated that **6** exists in a B type of conformer in $CDCl_3$ (Figure 4). In order to determine the absolute configuration of 1, Marfey's analysis[9] was appllied to *N*-methylserine produced by the acid hydrosis of **1**. The result revealed that the *N*-methylserine moiety of **1** has the L (*S*)-configuration. The above-summarized evidence led to absolute stereostructure (**1**) with the 3*S*-configuration for leptosin O.

Leptosin P (**2**) had the same molecular formula as **1** as deduced from HRSIMS. Its UV and IR spectra showed absorptions similar to those of **1**. The general features of the NMR spectra of the diacetate derivative (**7**) of **2** closely resembled those of **6** except that the ^{1}H NMR signal for the methyldithio group and some ^{13}C NMR signals (C-4, C-11, C-14, C-1', C-12' and C-14') exhibited a chemical shift difference relative to those of **6**, and the ^{1}H and ^{13}C NMR signals for one acetyl group were missing from **7** (Table 2). The presence of two acetoxy groups at C-11' and C-14', and the hydroxy group at C-12' was deduced from HMBC correlations (H-11'/11'-O$\underline{C}$OCH$_3$, H-14'/14'-O$\underline{C}$OCH$_3$, and 12'-OH/C-12'). In addition, NOESY (Table 2) of **7** exhibited NOE correlations similar to those of **6** except for NOEs from the methyldithio group to H-5a, H-5'a and 12'-OH. These observations showed **7** to be a stereoisomer of **6** at C-3, excluding the absence of 12'-OAc in **7**. (The relative configuration of **2** was thus established.) The same conformational analysis as above with **6** [δ 7.65 (H-10), δ 5.95 (H-10'); NOEs: H-10/H-11' and

Table 2 NMR spectral data of diacetate (**7**) of leptosin P (**2**) in $CDCl_3$

Position	δ_H[a]	*J* /Hz	^{1}H-^{1}H COSY	NOEs	δ_C	HMBC (C)[c]
1					157.16 (q)[b]	
3					84.72 (q)	
4					163.94 (q)	
5 a	5.34 s		6	6, 3-S_2CH_3, 5'a	78.52 (t)	4, 6a, 10a, 10'b
6	5.64 br s		5a	5a		5a, 6a, 10a
6 a					149.13 (q)	
7	6.61 d	7.6 (8)	8	8	109.64 (t)	6a, 9, 10a
8	7.08 t	7.6 (7, 9)	7, 9	7, 9	129.43 (t)	6a, 10
9	6.56 t	7.6 (8, 10)	8, 10	8, 10	118.49 (t)	7, 10a
10	7.65 d	7.6 (9)	9	9, 11'	127.02 (t)	6a, 8, 10a, 10b
10 a					123.31 (q)	
10 b					65.26 (q)	
11	6.73 s			5'a, 11', 12'-OH	119.86 (t)	1, 5a, 12, 10'b
12					132.33 (q)	
13	3.18 s			14	29.99 (p)	1, 3
14	2.62 heptet	6.8 (15, 16)	15, 16	13, 15, 16	37.88 (t)	3, 4, 15, 16
15	1.08 d	6.8 (14)	14	14, 16	18.06 (p)	3, 14
16	1.29 d	6.8 (14)	14	14, 15	18.33 (p)	3, 14
3-S_2CH_3	2.42 s			5a, 5'a, 12'-OH	22.95 (p)	
1'					164.45 (q)	
3'	4.19 dd	5.4 (14'A), 3.9 (14B')	14'A, 14'B	13', 14'A, 14' B	62.60 (t)	
4'					166.34 (q)	
5' a	5.92 br s		6'	5a, 11, 3-S_2CH_3, 14'A	77.25 (t)	4', 6'a, 10'a, 12'
6'	5.18 br s		5'a			6'a, 7, 10'a
6' a					149.29 (q)	
7'	6.47 d	7.6 (8')	8'	8'	109.59 (t)	6'a, 9', 10'a
8'	6.98 t	7.6 (7', 9')	7', 9'	9'	129.43 (t)	6'a, 10'
9'	6.78 t	7.6 (8', 10')	8', 10'	8', 10'	119.17 (t)	7', 10'a
10'	5.95 d	7.6 (9')	9'		126.54 (t)	8', 10'a, 10'b
10' a					125.11 (q)	
10' b					65.94 (q)	
11'	6.62 s			10, 11	79.84 (t)	10b, 1', 10'b, 12', 11'-O<u>C</u>OCH3
12'					86.87 (q)	
13'	2.92 s			3', 14'A, 14'B	32.53 (p)	1', 3'
14' A	4.53 dd	11.8 (14'B), 5.4 (3')	3'	3', 5'a, 13', 14'B	63.14 (s)	3', 4', 14'-O<u>C</u>OCH3
B	4.80 dd	11.8 (14'A), 3.9 (3')	3'	3', 13', 14'A		3', 4', 14'-O<u>C</u>OCH3
11'-OCOC<u>H</u>3	1.72 s				20.70 (p)	11'-O<u>C</u>OCH3
11'-O<u>C</u>OCH3					168.33 (q)	
12'-OH	4.72 br s			11, 3-S_2CH_3		12'
14'-OCOC<u>H</u>3	2.07 s				20.78 (p)	14'-O<u>C</u>OCH3
14'-O<u>C</u>OCH3					170.03 (q)	

a-c As in Table 1

H-11/H-5'a] demonstrated that **7** exists in a B type of conformer in $CDCl_3$ as in **6** (Figure 4). In addition to this evidence, application of the Marfey's analysis for the hydrolyzate of **2** led to absolute stereostructure (**2**) with the 3*R*-configuration for leptosin P.

Leptosin Q (**3**) had a molecular formula one sulfur atom less than that of **1** as deduced from HRSIMS. A

close inspection of its ^{1}H and ^{13}C NMR spectra of the triacetate derivative (**8**) of **3** revealed that the methyldithio group of **6** was replaced by a methylthio group in **8** (Tables 1 and 3). This was supported by HMBC correlations. Treatment of leptosn O (**1**) with $NaBH_4$ and CH_3I followed by Ac_2O in pyridine

Table 3 NMR spectral data of triacetates (**8**) and (**9**) in $CDCl_3$

Position	**8**			**9**		
	δ_H[a]	*J* /Hz	δ_C	δ_H[a]	*J* /Hz	δ_C
1			156.64 (q)[b]			155.72 (q)[b]
3			81.86 (q)			82.91 (q)
4			162.14 (q)			164.92 (q)
5 a	5.25 s		79.30 (t)	5.26 br s		79.21 (t)
6	5.48 br s			5.45 br s		
6 a			148.75 (q)			148.77 (q)
7	6.65 d	6.4 (8)	110.03 (t)	6.65 d	6.4 (8)	109.98 (t)
8	7.12 t	6.4 (7, 9)	129.59 (t)	7.13 t	6.4 (7, 9)	129.64 (t)
9	6.48 t	6.4 (8, 10)	118.86 (t)	6.46 t	6.4 (8, 10)	118.90 (t)
10	7.63 d	6.4 (9)	127.16 (t)	7.62 d	6.4 (9)	127.28 (t)
10 a			122.48 (q)			122.36 (q)
10 b			65.46 (q)			65.55 (q)
11	6.62 s		117.97 (t)	6.60 s		117.76 (t)
12			132.80 (q)			131.01 (q)
13	3.13 s		29.28 (p)	3.13 s		29.61 (p)
14	2.47 heptet	6.8 (15, 16)	36.26 (t)	2.40 heptet	6.8 (15, 16)	36.64 (t)
15	1.07 d	6.8 (14)	17.90 (p)	0.87 d	6.8 (14)	17.71 (p)
16	1.27 d	6.8 (14)	18.13 (p)	1.12 d	6.8 (14)	18.01 (p)
3-SCH_3	1.73 s		12.03 (p)	2.01 s		12.53 (p)
1'			160.33 (q)			160.32 (q)
3'	4.25 dd	6.0 (14'A), 2.8 (14B')	64.06 (t)	4.25 dd	6.8 (14'A), 2.8 (14'B)	64.08 (t)
4'			165.55 (q)			165.57 (q)
5' a	5.71 s		79.82 (t)	5.69 br s		79.74 (t)
6'	4.82 br s			4.85 br s		
6' a			148.88 (q)			148.90 (q)
7'	6.47 d	6.4 (8')	109.62 (t)	6.49 d	6.4 (8')	109.60 (t)
8'	7.09 t	6.4 (7', 9')	129.73 (t)	7.08 t	6.4 (7', 9')	129.64 (t)
9'	6.77 t	6.4 (8', 10')	119.53 (t)	6.74 t	6.4 (8', 10')	119.46 (t)
10'	5.87 d	6.4 (9')	125.29 (t)	5.88 d	6.4 (9')	125.24 (t)
10' a			126.52 (q)			126.50 (q)
10' b			64.78 (q)			64.78 (q)
11'	6.51 s		78.22 (t)	6.53 s		78.24 (t)
12'			89.50 (q)			89.50 (q)
13'	2.96 s		32.67 (p)	2.96 s		32.66 (p)
14' A	4.60 dd	12.2 (14'B), 6.0 (3')	65.77 (s)	4.59 dd	12.4 (14'B), 6.2 (3')	65.77 (s)
B	4.84 dd	12.2 (14'A), 2.8 (3')		4.83 dd	12.4 (14'A), 2.8 (3')	
11'-OCOC<u>H</u>3	1.77 s		20.62 (p)	1.79 s		20.64 (p)
11'-O<u>C</u>OCH3			167.58 (q)			167.60 (q)
12'-OCOC<u>H</u>3	2.26 s		21.14 (p)	2.26 s		21.20 (p)
12'-O<u>C</u>OCH3			170.33 (q)			170.35 (q)
14'-OCOC<u>H</u>3	2.10 s		20.90 (p)	2.10 s		20.90 (p)
14'-O<u>C</u>OCH3			170.16 (q)			170.15 (q)

a-c As in Table 1

provided the triacetate, which was identical with **8** as judged by comparison of spectral data and specific optical rotations. This evidence led to absolute stereostructure (**3**) for leptosin Q. The triacetate (**8**) also was shown to exist in a B type of conformer in $CDCl_3$ on the basis of the same conformational analysis as above with **6** (Figure 4).

Leptosin R (**4**) had the same molecular formula as **3** as deduced from HRSIMS. The general features of

Table 4 NMR spectral data of tetraacetate (**10**) of leptosin S (**5**) in $CDCl_3$

Position	δ_H[a]	*J* /Hz	^{1}H-^{1}H COSY	NOEs	δ_C	HMBC (C)[c]
1					157.00 (q)[b]	
3					77.28 (q)	
4					161.07 (q)	
5 a	5.47 s			5'a, 11'	79.08 (t)	4, 6a, 10a, 11, 12, 10'b
6	4.53 br s					6a, 5a
6 a					149.47 (q)	
7	6.46 d	7.6 (8)	8	8	109.97 (t)	6a, 9, 10a
8	7.10 t	7.6 (7, 9)	7, 9	7, 9	129.54 (t)	6a, 7, 10
9	6.86 t	7.6 (8, 10)	8, 10	8, 10	119.67 (t)	7, 8, 10a
10	7.74 d	7.6 (9)	9	11'	125.38 (t)	6a, 8, 10a, 10b
10 a					121.54 (q)	
10 b					65.43 (q)	
11	6.72 s			5'a, 12'-OCOC<u>H</u>$_3$	117.23 (t)	1, 5a, 12, 10b
12					133.82 (q)	
13	2.86 s			14, 15, 16	28.00 (p)	1, 3
14	2.20 heptet	6.6 (15, 16)	15, 16	13, 15, 16	35.15 (t)	3, 4, 15, 16
15	1.05 d	6.6 (14)	14	13, 14, 15, 5'a	16.83 (p)	3, 14, 15
16	0.82 d	6.6 (14)	14	13, 14, 16	16.63 (p)	3, 14, 16
3-N<u>H</u>COCH$_3$	6.18 s					3, 4, 14
3-NHCOC<u>H</u>$_3$	2.06 s				22.81 (p)	3-NH<u>C</u>OCH$_3$
3-NH<u>C</u>OCH$_3$					170.14 (q)	
1'					160.73 (q)	
3'	4.22 dd	5.8 (14'A), 2.8 (14B')	14'A, 14'B	13', 14'B	64.07 (t)	1', 4', 14'
4'					164.93 (q)	
5' a	5.93 s			5a, 16, 11', 14'A 12'-OCOC<u>H</u>$_3$	79.52 (t)	4', 6'a, 12'
6'	5.85 br s					5'a, 7, 10'a
6' a					148.95 (q)	
7'	6.61 d	7.6 (8')	8'	8'	109.58 (t)	6'a, 9
8'	7.04 t	7.6 (7', 9')	7', 9'	7', 9'	129.23 (t)	6'a, 7', 10'
9'	6.37 t	7.6 (8', 10')	8', 10'	8', 10'	118.29 (t)	7', 10'a
10'	5.68 d	7.6 (9')	9'		127.29 (t)	8', 9', 10'b
10' a					122.30 (q)	
10' b					65.06 (q)	
11'	6.62 s			10, 11	78.24 (t)	10b, 1', 10'b, 12', 11'-O<u>C</u>OCH$_3$
12'					89.35 (q)	
13'	2.96 s			3', 14'A, 14'B	32.69 (p)	1', 3'
14' A B	4.64 dd 4.81 dd	12.3 (14'B), 5.8 (3') 12.3 (14'A), 2.8 (3')	3' 3'	5'a, 13', 14'B 3', 13', 14'A	66.11 (s)	4', 14'-O<u>C</u>OCH$_3$ 3', 4', 14'-O<u>C</u>OCH$_3$
11'-OCOC<u>H</u>$_3$	1.74 s				20.65 (p)	11'-O<u>C</u>OCH$_3$
11'-O<u>C</u>OCH$_3$					167.65 (q)	
12'-OCOC<u>H</u>$_3$	2.27 s			11, 5'a	21.07 (p)	12'-O<u>C</u>OCH$_3$
12'-O<u>C</u>OCH$_3$					170.20 (q)	
14'-OCOC<u>H</u>$_3$	2.09 s				20.94 (p)	14'-O<u>C</u>OCH$_3$
14'-O<u>C</u>OCH$_3$					170.57 (q)	

a-c As in Table 1

the NMR spectra of the triacetate derivative (**9**) of **4** closely resembled those of **8** except that the ^{1}H NMR signals for the methylthio and isopropyl groups and the ^{13}C NMR signals for C-1, C-3, C-4 and C-12 in **9** exhibited a chemical shift difference relative to those of **8** (Table 3). This evidence and HMBC correlations of **9** suggested that **9** is a stereoisomer of **8** at C-3. Treatment of **2** with $NaBH_4$ and CH_3I followed by Ac_2O in pyridine provided the triacetate, identical with **9**. Based on this evidence, the absolute stereostructure (**4**) for leptosin R was elucidated. The triacetate (**9**) also was shown to exist in a B type of conformer in $CDCl_3$ on the basis of the same conformational analysis as above with **6** (Figure 4). Leptosin S (**5**) was assigned the molecular formula $C_{32}H_{35}N_7O_7$ deduced from HRSIMS. A close inspection of its ^{1}H and ^{13}C NMR spectra of the tetraacetate derivative (**10**) of **5** revealed that the methylthio group in **8** or **9** was replaced by a secondary actamide group [δ_H 2.06 ($COCH_3$), 6.18 (NHCO); δ_C 22.81 ($CO\underline{C}H_3$), 170.14 ($\underline{C}OCH_3$)] in **10** (Tables 3 and 4). This was supported by HMBC correlations. NOESY (Table 5) of **10** exhibited NOE correlations from H-5'a to H-5a, H-15, H-14' and 12'-OAc, and from H-11' to H-11 and 12'-OAc, implying that the relative configuration of **10** is the same as **6** and **8**. The tetraacetate (**10**) also was shown to exist in a B type of conformer in $CDCl_3$ on the basis of the same conformational analysis as above with **6** (Figure 4). In addition to the above evidence, application of the Marfey's analysis for the hydrolyzate of **5** led to absolute stereostructure (**5**) with the 3*R*-configuration for leptosin S.

In the CD spectra, leptosin O (**1**) with the 3*S*-stereoconfiguration exhibited the different curve from leptosin P (**2**) with the 3*R*-stereoconfiguration (Figure 5). The CD curves of leptosins Q (**3**) and S (**5**) closely resembled that of **1**, while leptosin R (**4**) exhibited the similar curve with that of **2**. This fact suggests that there is the possibility of utilizing CD spectra in order to decide the 3-stereoconfiguration of leptosins O (**1**)–S (**5**).

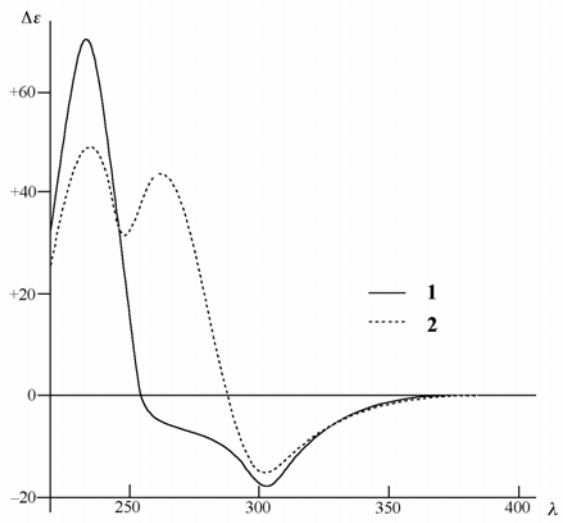


Figure 5 CD spectra of leptosin O (**1**) and P (**2**)

The cancer cell growth inhibitory properties of leptosins were examined using the murine P388 lymphocytic leukemia cell line[4] and a disease-oriented panel of 39 human cancer cell lines (HCC panel) in the Japanese Foundation for Cancer Research.[10] Among these metabolites (**1**–**5**), leptosins O (**1**) and P (**2**) with the methyldithio group exhibited significant cytotoxic activity against the murine P388 cell line (Table 5), and the activity of **2** were almost 10-fold potent than

Table 5 Cytotoxity of leptosins O-P (**1**-**5**) against P388 cells (DMSO was used for vehicle)

	Compound	ED_{50} (μg/mL)
Leptosin	O (**1**)	1.1
	P (**2**)	0.1
	Q (**3**)	14.8
	R (**4**)	15.2
	S (**5**)	10.1
5-FU	(standard)	5.8 x 10^{-2}

those of **1**. In addition, leptosin O (**1**) and S (**5**) showed moderate cytotoxic activities against the 39 human cancer cell lines (Mean value of log GI_{50} over all cell lines: –4.01 and –4.01 M, respectively; The delta value: 0.38 and 0.12, respectively; The range value: 0.39 and 0.13, respectively).

EXPERIMENTAL

General procedures

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu spectrophotometer, IR spectra on a Perkin Elmer FT-IR spectrometer 1720X and CD spectra on a JASCO J-500A spectrometer. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. NMR spectra were recorded at 27ºC on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, with tetramethylsilane (TMS) as an internal reference. SIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed in a medium pressure. Preparative HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm x 20 mm i. d.). Analytical HPLC was run on a Waters 484 instrument equipped with a photodiode array detector (900J) and Cosmosil $5C_{18}$-MS (15 cm x 4.6 mm i. d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent system CH_2Cl_2-MeOH (19:1), and compounds were viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Culturing and isolation of metabolites

As reported previously,[4] a strain of *Leptosphaeria* sp. OUPS-N80 was isolated from the marine alga *Sargassum tortile*. The fungal strain was grown in a liquid medium (90 L) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5 for 3 weeks at 27ºC. The MeOH extract (52.3 g) of the mycelium was dissolved in CH_2Cl_2–MeOH (1 : 1), and the soluble fraction (32.7 g) was passed through Sephadex LH-20, using CH_2Cl_2–MeOH (1 : 1) as the eluent. The second fraction (12.8 g), in which the activity was concentrated, was chromatographed on a silica gel column with a hexane–CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (2 : 98) eluate (211.7 mg) was further purified by HPLC using MeOH–H_2O (8 : 2) as the eluent to afford **1** (18.8 mg) and **2** (17.2 mg). The MeOH–CH_2Cl_2 (5 : 95) eluate (426.6 mg) afforded **5** (22.6 mg) after purification by HPLC using MeOH–H2O (7 : 3) as the eluent. Using the same procedure as above with the AcOEt extract (3.7 g) of the culture filtralte, the MeOH–CH_2Cl_2 (2 : 98) eluate (73.5 mg) on a silica gel column was purified by HPLC using MeOH–H_2O (8 : 2) as the eluent to afford afford **3** (7.2 mg) and **4** (6.8 mg).

Leptosin O (1). Obtained as a pale yellow powder, mp 220–222ºC, $[\alpha]_D$ –99º (*c* 0.08, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 211 (4.76), 238 (4.58), 265 (4.30), 298 (4.08). IR ν_{max} (KBr) cm^{-1} : 3524 (NH, OH), 1688, 1658 (amide) 1610, 1599 (Ar.). SIMS *m/z* (rel. int.) : 693 ($[M+H]^+$, 7.1%), 613 (M^+–S_2Me, 47.3), 296 ($[C_{17}H_{18}N_3O_2]^+$, 100), 232 (bis-indol-3-yl, 37.2). HRSIMS *m/z* : 693.2166 $[M+H]^+$ (calcd for $C_{33}H_{37}N_6O_7S_2$: 693.2164). ^{1}H NMR δ ppm (acetone-d_6) : 1.06 (3H, d, *J* = 6.8 Hz, H-15), 1.27 (3H, d, *J* = 6.8 Hz, H-16), 2.20 (3H, s, 3-S_2Me), 2.52 (1H, heptet, *J* = 6.8 Hz, H-14), 2.90 (3H, s, H-13), 3.06 (3H, s, H-13'), 3.84 (1H, br s, H-3'), 4.00 (2H, br s, H-14'), 5.20 (1H, br s, 11'-OH), 5.29 (1H, br s, H-5a), 5.69 (1H, br s, H-6), 6.69 (1H, s, H-11). ^{13}C NMR δ ppm (acetone-d_6) : 18.09 (C-15), 18.29 (C-16), 22.86 (3-S_2Me), 29.53 (C-13), 31.74 (C-13'), 35.62 (C-14), 59.54 (C-14'), 64.92, 65.71 (C-3'), 66.13, 78.41 (C-5'a), 79.11 (C-5a), 80.18 (C-11'), 84.08 (C-3), 86.81 (C-12'), 109.67, 109.89, 118.47, 122.69,

129.81, 133.54 (C-12), 149.51, 157.22 (C-1), 160.41 (C-4'), 165.68 (C-4), 167.60 (C-1'). CD λ nm ($\Delta\varepsilon$) [*c* 2.29$\times$ 10^{-5}, EtOH] : 384 (0), 301 (–19.11), 275 (–7.94), 261 (0), 234 (+70.13).

Leptosin P (2). Obtained as a pale yellow powder, mp 233–235ºC, $[\alpha]_D$ +35º (*c* 0.17, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 209 (4.49), 238 (4.30), 270 (3.99), 300 (3.78). IR ν_{max} (KBr) cm^{-1} : 3504 (NH, OH), 1690, 1660 (amide), 1608, 1595 (Ar.). SIMS *m/z* (rel. int.) : 693 ($[M+H]^+$, 3.6%), 613 (M^+–S_2Me, 53.2), 296 ($[C_{17}H_{18}N_3O_2]^+$, 100), 232 (bis-indol-3-yl, 23.0). HRSIMS *m/z* : 693.2166 $[M+H]^+$ (calcd for $C_{33}H_{37}N_6O_7S_2$: 693.2164). ^{1}H NMR δ ppm (acetone-d_6) : 1.00 (3H, d, *J* = 6.8 Hz, H-15), 1.26 (3H, d, *J* = 6.8 Hz, H-16), 2.43 (3H, s, 3-S_2Me), 2.48 (1H, heptet, *J* = 6.8 Hz, H-14), 2.88 (3H, s, H-13'), 3.07 (3H, s, H-13), 3.85 (1H, br s, H-3'), 3.99 (1H, dd, *J* = 11.8, 3.1 Hz, H-14'A), 4.11 (1H, dd, *J* = 11.8, 3.1 Hz, H-14'B), 5.39 (1H, br s, H-5a), 6.67 (1H, s, H-11). ^{13}C NMR δ ppm (acetone-d_6) : 18.14 (C-15), 18.37 (C-16), 22.94 (3-S_2Me), 29.70 (C-13), 31.87 (C-13'), 35.91 (C-14), 59.77 (C-14'), 65.23, 65.48 (C-3'), 65.55, 77.98, 79.40, 79.98 (C-11'), 84.48 (C-3), 86.77 (C-12'), 109.61, 109.76, 118.41, 118.98, 119.19, 127.38, 129.60, 129.73, 149.28, 149.38, 157.51 (C-1), 161.85 (C-4'), 166.86 (C-4), 167.81 (C-1'). CD λ nm ($\Delta\varepsilon$) [*c* 4.96$\times10^{-5}$, EtOH] : 385 (0), 303 (–16.72), 289 (0), 268 (+41.91), 235 (+47.11).

Leptosin Q (3). Obtained as a pale yellow powder, mp 231–233ºC, $[\alpha]_D$ –92º (*c* 0.07, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 212 (4.76), 238 (4.32), 268 (4.02), 299 (3.75). IR ν_{max} (KBr) cm^{-1}: 3505 (NH, OH), 1686, 1664 (amide) 1610, 1579 (Ar.). SIMS m/z (rel. int.) : 660 ($[M]^+$, 21.5%), 613 (M^+–SMe, 78.6), 296 ($[C_{17}H_{18}N_3O_2]^+$, 100), 232 (bis-indol-3-yl, 13.1). HRSIMS *m/z* : 660.2365 $[M]^+$ (calcd for $C_{33}H_{37}N_6O_7S$: 660.2367). ^{1}H NMR δ ppm (acetone-d_6) : 1.04 (3H, d, *J* = 6.8 Hz, H-15), 1.23 (3H, d, *J* = 6.8 Hz, H-16), 1.87 (3H, s, 3-SMe), 2.38 (1H, heptet, *J* = 6.8 Hz, H-14), 2.90 (3H, s, H-13'), 3.17 (3H, s, H-13), 3.83 (1H, br s, H-3'), 3.99 (2H, br s, H-14'), 5.29 (1H, br s, H-5a), 6.62 (1H, s, H-11), 7.05 (1H, br s, H-8'), 7.20 (1H, br s, H-8), 7.60 (1H, br s, H-10). ^{13}C NMR δ ppm (acetone-d_6) : 12.31 (3-SMe), 17.68 (C-15), 18.22 (C-16), 29.70 (C-13), 32.07 (C-13'), 36.21 (C-14), 59.50 (C-14'), 64.86, 65.63 (C-3'), 79.24, 81.48 (C-3), 86.72 (C-12'), 109.66, 109.89, 118.44, 118.87, 124.96, 129.23, 129.47, 129.85, 149.39, 157.35 (C-1), 161.60 (C-4'). CD λ nm ($\Delta\varepsilon$) [c 1.67$\times10^{-5}$, EtOH] : 382 (0), 299 (–19.02), 275 (–10.52), 259 (0), 231 (+65.46).

Leptosin R (4). Obtained as a pale yellow powder, mp 221–223ºC, $[\alpha]_D$ –24º (*c* 0.10, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 212 (4.95), 238 (4.72), 267 (4.35), 298 (4.14). IR ν_{max} (KBr) cm^{-1} : 3504 (NH, OH), 1687, 1664 (amide), 1611, 1579 (Ar.). SIMS m/z (rel. int.) : ($[M]^+$, 19.3%), 613 (M^+–SMe, 65.6), 296 ($[C_{17}H_{18}N_3O_2]^+$, 100), 232 (bis-indol-3-yl, 15.8). HRSIMS m/z : 660.2365 $[M]^+$ (calcd for $C_{33}H_{37}N_6O_7S$: 660.2367). ^{1}H NMR δ ppm (acetone-d_6) : 0.90 (3H, d, *J* = 6.8 Hz, H-15), 1.18 (3H, d, *J* = 6.8 Hz, H-16), 2.00 (3H, s, 3-SMe), 2.42 (1H, heptet, *J* = 6.8 Hz, H-14), 2.91 (3H, s, H-13'), 3.16 (3H, s, H-13), 3.85 (1H, br s, H-3'), 4.01 (1H, d, *J* = 9.8 Hz, H-14'A), 4.10 (1H, d, *J* = 9.8 Hz, H-14'B), 5.33 (1H, br s, H-5a), 6.63 (1H, s, H-11), 7.05 (1H, br s, H-8'), 7.18 (1H, br s, H-8). ^{13}C NMR δ ppm (acetone-d_6) : 12.00 (3-SMe), 17.76 (C-15), 18.27 (C-16), 29.76 (C-13), 31.80 (C-13'), 36.61 (C-14), 59.64 (C-14'), 64.99, 65.49 (C-3'), 79.40, 82.02 (C-3), 86.60 (C-12'), 109.52, 109.77, 118.45, 118.82, 125.01, 129.23, 129.56, 129.81, 149.22, 157.29 (C-1), 161.41 (C-4'). CD λ nm ($\Delta\varepsilon$) [*c* 1.38$\times10^{-5}$, EtOH] : 386 (0), 304 (–18.27), 292 (0), 266 (+40.26), 237 (+48.74).

Leptosin S (5). Obtained as a pale yellow powder, mp 231–233ºC, $[\alpha]_D$ –56º (*c* 0.07, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 212 (4.77), 238 (4.61), 270 (4.10), 300 (4.04). IR ν_{max} (KBr) cm^{-1} : 3526 (NH, OH), 1677, 1666 (amide), 1607, 1575 (Ar.). SIMS *m/z* (rel. int.) : 629 ($[M]^+$, 3.0%), 613 (M^+–NH_2, 43.6), 296 ($[C_{17}H_{18}N_3O_2]^+$, 100), 232 (bis-indol-3-yl, 91.7). HRSIMS *m/z* : 629.2596 $[M]^+$ (calcd for $C_{32}H_{35}N_7O_7$: 629.2599). ^{1}H NMR δ ppm (acetone-d_6) :

0.69 (3H, d, J = 6.8 Hz, H-15), 0.96 (3H, d, J = 6.8 Hz, H-16), 2.07 (1H, heptet, J = 6.8 Hz, H-14), 2.90 (3H, s, H-13'), 3.04 (3H, s, H-13), 3.85 (1H, br s, H-3'), 4.01 (1H, dd, J = 10.8, 3.8 Hz, H-14'A), 4.05 (1H, dd, J = 11.8, 3.1 Hz, H-14'B), 5.45 (1H, br s, H-5a), 6.72 (1H, s, H-11). ^{13}C NMR δ ppm (acetone-d_6) : 16.65 (C-15), 16.65 (C-16), 28.00 (C-13'), 31.48 (C-13), 38.00 (C-14), 59.65 (C-14'), 66.02 (C-3'), 79.12 (C-5'a), 79.51 (C-5a), 80.28 (C-11'), 87.23 (C-12'), 109.60, 110.11, 118.31, 119.68, 123.15, 125.33, 127.30, 127.71, 129.26, 129.60, 134.00, 149.10, 150.03, 157.18 (C-1), 164.22, 167.25, 169.10 (C-1'). CD λ nm ($\Delta\varepsilon$) [c 2.11$\times10^{-5}$, EtOH] : 383 (0), 301 (–17.96), 275 (–7.98), 262 (0), 232 (+64.98).

Formation of acetate

The triacetate 6 from leptosin O (1). Leptosin O (**1**) (8.2 mg) was dissolved in a solution (0.5 mL) of pyridine. To the solution were added Ac_2O (0.5 mL), and the mixture was left at rt overnight. The reaction mixture was then evaporated off under reduced pressure, and the residue was purified by HPLC using MeOH–H_2O (8 : 2) as the eluent to afford **6** (7.2 mg) as a pale yellow powder. mp 212–214ºC, $[\alpha]_D$ –33º (c 0.08, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 211 (4.76), 237 (4.57), 265 (4.30), 298 (4.12). IR ν_{max} (KBr) cm^{-1} : 3525 (NH), 1725 (ester), 1686, 1658 (amide), 1611,1597 (Ar.). SIMS m/z (rel. int.) : 819 ([M+H]$^+$, 3.2%), 639 (MH$^+$–3AcOH, 45.7), 296 ([$C_{17}H_{18}N_3O_2$]$^+$, 100). HRSIMS m/z : 819.2471 [M+H]$^+$ (Calcd for $C_{39}H_{43}N_6O_{10}S_2$: 819.2471). CD λ nm ($\Delta\varepsilon$) [c 1.26$\times10^{-5}$ M, EtOH] : 386 (0), 301 (–18.76), 275 (–8.11), 262 (0), 233 (+69.18). ^{1}H and ^{13}C NMR spectral data are listed in Table 1.

The diacetate 7 from leptosin P (2). Using the same procedure as above with compound (**1**), leptosins P (**2**) (7.3 mg) was treated with dry pyridine (0.5 mL) and Ac_2O (0.5 mL), and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **8** (5.6 mg) as a pale yellow powder. mp 228–230ºC, $[\alpha]_D$ +20º (c 0.26, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 210 (4.50), 238 (4.32), 268 (4.00), 300 (3.69). IR ν_{max} (KBr) cm^{-1} : 3508 (NH), 1730 (ester), 1688, 1662 (amide), 1608, 1598 (Ar.). SIMS m/z (rel. int.) : 777 ([M+H]$^+$, 0.3%), 639 (MH$^+$–2AcOH–H_2O, 3.8), 296 ([$C_{17}H_{18}N_3O_2$]$^+$, 100). HRSIMS m/z : 777.2374 [M+H]$^+$ (Calcd for $C_{37}H_{41}N_6O_9S_2$: 777.2393). CD λ nm ($\Delta\varepsilon$) [c 1.26$\times10^{-5}$ M, EtOH] : 388 (0), 305 (–15.61), 290 (0), 266 (+43.46), 238 (+48.54). ^{1}H and ^{13}C NMR spectral data are listed in Table 2.

The triacetate 8 from leptosin Q (3). Using the same procedure as above with compound (**1**), leptosins Q (**3**) (5.5 mg) was treated with pyridine (0.4 mL) and Ac_2O (0.4 mL), and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **8** (4.8 mg) as a pale yellow powder. mp 218–220ºC, $[\alpha]_D$ –65º (c 0.08, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 212 (4.75), 237 (4.33), 268 (4.02), 298 (3.71). IR ν_{max} (KBr) cm^{-1} : 3512 (NH), 1721 (ester), 1686, 1665 (amide), 1611, 1580 (Ar.). SIMS m/z (rel. int.) : ([M+H]$^+$, 2.1%), 547 (MH$^+$–3AcOH, 47.8), 296 ([$C_{17}H_{18}N_3O_2$]$^+$, 100). HRSIMS m/z : 727.2753 [M+H]$^+$ (Calcd for $C_{39}H_{43}N_6O_{10}S$: 727.2755). CD λ nm ($\Delta\varepsilon$) [c 2.19$\times10^{-5}$ M, EtOH] : 380 (0), 299 (–20.11), 275 (–10.05), 260 (0), 231 (+64.92). ^{1}H and ^{13}C NMR spectral data are listed in Table 3.

The triacetate 9 from leptosin R (4). Using the same procedure as above with compound (**1**), leptosins R (**4**) (5.8 mg) was treated with pyridine (0.4 mL) and Ac_2O (0.4 mL) and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **9** (4.5 mg) as a pale yellow powder. mp 215–217ºC, $[\alpha]_D$ –18º (c 0.09, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 212 (4.86), 238 (4.68), 266 (4.30), 298 (4.07). IR ν_{max} (KBr) cm^{-1} : 3507 (NH), 1725 (ester), 1688, 1664 (amide), 1610, 1580 (Ar.). SIMS m/z (rel. int.) : 727 ([M+H]$^+$, 0.8%), 547 (MH$^+$–3AcOH, 53.3), 296 ([$C_{17}H_{18}N_3O_2$]$^+$, 100). HRSIMS m/z : 727.2753 [M+H]$^+$ (Calcd for $C_{39}H_{43}N_6O_{10}S$: 727.2759). CD λ nm ($\Delta\varepsilon$) [c 2.60

$\times 10^{-5}$ M, EtOH] : 386 (0), 304 (–17.96), 291 (0), 266 (+41.33), 237 (+46.92). ^{1}H and ^{13}C NMR spectral data are listed in Table 3.

The tetraacetate 10 from leptosin S (5). Using the same procedure as above with compound (**1**), leptosins S (**5**) (7.2 mg) was treated with pyridine (0.4 mL) and Ac_2O (0.4 mL), and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **10** (6.2 mg) as a pale yellow powder. mp 218–220ºC, $[\alpha]_D$ –24º (*c* 0.11, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε) : 211 (4.76), 238 (4.62), 270 (4.18), 300 (3.98). IR ν_{max} (KBr) cm^{-1} : 3525 (NH), 1722 (ester), 1678, 1666 (amide), 1607, 1575 (Ar.). SIMS *m/z* (rel. int.) : 797 ([M+H]$^+$, 3.2%), 523 (MH$^+$–4AcOH–H_2O–NH, 64.3), 294 ([$C_{17}H_{16}N_3O_2$]$^+$, 100). HRSIMS *m/z* : 797.3016 [M+H]$^+$ (Calcd for $C_{40}H_{43}N_7O_{11}$: 797.3014). CD λ nm ($\Delta\varepsilon$) [*c* 3.25$\times 10^{-5}$ M, EtOH] : 380 (0), 302 (–16.58), 276 (–8.11), 260 (0), 233 (+65.18). ^{1}H and ^{13}C NMR spectral data are listed in Table 4.

The triacetate 8 from leptosin O (1). Leptosin O (**1**) (5.3 mg) was dissolved in a solution (0.4 mL) of pyridine and MeOH (5 : 8). To the solution were added MeI (1.0 mL) and $NaBH_4$ (3.0 mg), and the mixture was stirred for 20 min at rt. The reaction mixture was then diluted with water and extracted with diethyl ether. The ether layer was evaporated off under reduced pressure, and the residue was purified by HPLC using MeOH–H_2O (8 : 2) as the eluent to afford **3** (2.1 mg) as a pale yellow powder. Therefore, using acetylation as above with compound (**1**), this compound was treated with pyridine (0.4 mL) and Ac_2O (0.4 mL) and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **8** (2.1 mg) as a pale yellow powder.

The triacetate 9 from leptosin P (2). Using the same procedure as above, a solution (0.3 mL) of Leptosin P (**2**) (5.8 mg) in pyridine/MeOH (5 : 8) was treated with MeI (1.0 mL) and $NaBH_4$ (3.0 mg), and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **4** (2.2 mg) as a pale yellow powder. Therefore, this compound was treated with pyridine (0.4 mL) and Ac_2O (0.4 mL) and the product was purified by HPLC [MeOH–H_2O (8 : 2)] to afford **9** (1.8 mg) as a pale yellow powder.

Marfey analyses of *N*-methylserine produced from leptosins O (1), P (2) and S (5).

As reported previously,[9] leptosins O (**1**) (1.2 mg), P (**2**) (1.5 mg) and S (**5**) (1.3 mg) were dissolved in MeOH (0.2 mL) and 6 N HCl (2 mL) and sealed in separate vials. The vials were heated at 110 C for 36 h, and the solution was evaporated *in vacuo*. To the acid hydrolyzates of **1**, **2** and **5** were added a solution of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA) in acetone (100 μL) and 1 M $NaHCO_3$ (100 μL). The mixtures were heated at 80ºC for 30 min followed by neutralization with 2 N HCl (50 μL) and evaporated off under reduced pressure. The residues were dissolved in 50 % aqueous MeOH and subjected to reversed phase HPLC: Cosmosil 5C_{18}-MS (15 cm x 4.6 mm i. d.), MeOH–H_2O–TFA (30 : 70 : 0.1) as the eluent, flow rate 1 mL/min, UV detection 340 nm. *N*-Me-L-Ser-L-FDAA derivative was detected from all of the acid hydrolyzates of **1**, **2** and **5**; retention time (min): *N*-Me-D-Ser-L-FDAA (55.2), *N*-Me-L-Ser-L-FDAA (56.8). The identity of the peak was confirmed by co-injection with a solution of a standard derivatized in the same manner.

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