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Potent Cytotoxic Metabolites from a *Leptosphaeria* Species. Structure Determination and Conformational Analysis

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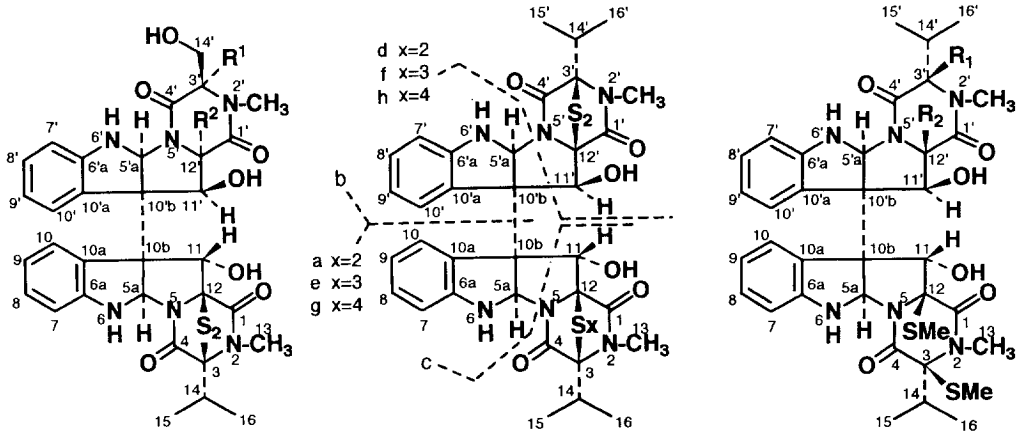
Abstract: New cytotoxic epipolysulfanyldioxopiperazine dimers, leptosins K (**4**), K_1 (**5**) and K_2 (**6**), have been isolated from a strain of *Leptosphaeria* sp. originally isolated from the marine alga *Sargassum tortile*. Their stereostructures, with a different configuration from that of leptosins A–C, have been elucidated by spectral and X-ray analyses and some chemical transformations. X-Ray and NMR and NOE spectral analyses of **4** revealed that it exists in a mixture of four conformers, of which two each closely resemble, in a single crystal, and in a single conformer in solution. NOE experiments of **5** and **6** demonstrated that they exist in a mixture of two conformers slowly exchanging in $CDCl_3$.

Based on the fact that some of bioactive materials isolated from marine animals have been produced by bacteria,[1-4] we have focused our attention on new antitumour and cytotoxic materials from microorganisms inhabiting the marine environment. As part of this program, we reported that antitumour and cytotoxic compounds, leptosins A (**1**), B (**2**) and C (**3**) – J, belonging to a series of epipolysulfanyldioxopiperazines, were produced by a strain of *Leptosphaeria* sp. OUPS-4 isolated from the marine alga *Sargassum tortile* C. AGAROH (Sargassaceae).[5-7] Our continuing search for cytotoxic metabolites from this fungal strain led to the isolation of three new epipolysulfanyldioxopiperazine dimers designated leptosins K (**4**), K_1 (**5**) and K_2 (**6**), which exhibited potent cytotoxic activity in the P388 lymphocytic leukemia test system in cell culture. NMR spectral analyses using heteronuclear multiple-bond connectivity (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments, and an X-ray crystallographic analysis revealed that compounds **4–6** have dimeric epipolysulfanyldioxopiperazine skeletons with a different configuration from that of **1–3** [5]. The X-ray analysis of **4** demonstrated that it exists in a mixture of four conformers, of which two each closely resemble, in a single crystal. NMR and NOE spectral analyses for solution conformation of leptosins showed that **4**, and **5** and **6** exist in a single conformer and in a mixture of two conformers in $CDCl_3$ solution, respectively, but all of them exist in a single conformer in pyridine-d_5. Herein we report the structure determination of **4–6**, their conformations and cytotoxic activities.

Results and Discussion

From an MeOH extract of the mycelium of the fungal strain, three leptosins **4–6** were isolated by a combination of LH-20 and silica gel column chromatographies and high-performance liquid chromatography (HPLC).

Leptosin K (**4**) had the molecular formula $C_{34}H_{36}N_6O_6S_4$ established by high-resolution fast atoms bombardment mass spectrometry (HRFABMS). Its IR spectrum exhibited bands characteristic of an alcohol,



1 $R^1 = R^2 = S_4$ **3** $R^1 = R^2 = S_2$
2 $R^1 = R^2 = S_3$ **12** $R^1 = R^2 =$ SMe

4 x = 2
5 x = 3
6 x = 4

7 $R^1 = R^2 = -S_2-$
8 $R^1 = R^2 =$ SMe

an amine, an amide and an aromatic ring. A close inspection of the ^1H- and ^{13}C NMR spectra of **4** in $CDCl_3$ (Table 1) by distortionless enhancement by polarization transfer (DEPT) and ^1H–^1H and ^1H–^{13}C correlation spectroscopy (COSY) experiments and comparison with spectral data for **1**–**3** revealed signals for the following functionalities : two hydroxy methine groups (C-11 and C-11') each linked to two quaternary sp^3-hybridized carbons, two methines (C-5a and C-5'a) bearing two nitrogens and a quaternary sp^3-carbon, four quaternary sp^3-carbons (C-3, C-12, C-3' and C-12') each bearing a nitrogen and a sulfur, four amides (C-1, C-4, C-1' and C-4'), two N-methyl groups (C-13 and C-13'), two isopropyl groups (C-14 to C-16 and C-14' to C-16') each linked to a quaternary sp^3- carbon, 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, and two benzylic quaternary sp^3-carbons (C-10b and C-10'b).

Treatment of **4** with $NaBH_4$ and MeI afforded bis(methylsulfanyl) and tetrakis(methylsulfanyl) derivatives **7** and **8**, which had the molecular formulae $C_{36}H_{42}N_6O_6S_4$ and $C_{38}H_{48}N_6O_6S_4$ established by $[M+1]^+$ peaks in FABMS, respectively. The ^1H and ^{13}C NMR spectra of **8** in $CDCl_3$ (Table 2) indicated the presence of four S-methyl groups besides the functionalities corresponding to those of **4**. HMBC correlations (Table 2) allowed the connection of the functional groups of **8** and led to the planar structure of **8** for the tetrakis(methylsulfanyl) derivative. The principal HMBC correlations are as follows: 5a-H to C-4 and C-10'b, 11-H to C-5a and C-10a, 13-H to C-1 and C-3, 14-H to C-4, 15-H to C-3, 5'a-H to C-10b, C-6'a and C-12', 11'-H to C-10b, C-1' and C-10'a, 13'-H to C-1' and C-3', 14'-H to C-4', 15'-H to C-3', and protons for four S-Me to C-3, C-12, C-3' and C-12' each. Formation of **8** from **4** and the molecular formula of **4** demonstrated that a disulfide bridge exists in each of two isopropyl-bearing dioxopiperazine rings of **4**, and led to planar structure **4** for leptosin K. This structure was supported by the FABMS fragment at *m/z* 376 (a^+ or b^+), corresponding to one of two equivalent monomeric subunits, as well as three other ions at *m/z* 312 ([a or b $-2S]^+$), 493 ([c or d $+H]^+$) and 428 ([c or d $-2S]^+$). Incidentally, assignments of ^1H and ^{13}C NMR signals for **4** were based on the application of a long range ^1H–^{13}C COSY experiment and comparison with spectral data for **8** assigned by HMBC correlations (Tables 1 and 2).

Table 1 ^{1}H and ^{13}C NMR spectral data of leptosin K (**4**) in $CDCl_3$ and pyridine-d_5

	$CDCl_3$					pyridine-d_5			
position	δ ^{1}H[a]	NOEs (H)[b]	δ ^{13}C		LR ^{1}H–^{13}C correlation (H)[d]	δ ^{1}H	NOEs (H)[b]	δ ^{13}C	
1			167.45	(q)[c]	13			166.66	(q)
3			80.25	(q)	13			81.90	(q)
4			160.56	(q)				161.01	(q)
5a	5.98 s	5'a, 11'	78.84	(t)	11	6.62 s	5'a	80.44	(t)
6	6.80 s					7.57 br s			
6a			148.02	(q)	5a			152.26	(q)
7	6.62 d (7.8)		110.21	(t)		6.65 d (7.6)		109.52	(t)
8	7.09 t (7.8)		130.17	(t)		7.33 t (7.6)		130.26	(t)
9	6.45 t (7.8)		119.74	(t)		7.04 t (7.6)		117.57	(t)
10	5.75 t (7.8)	11	125.17	(t)		8.14 d (7.6)	11, 11'	127.03	(t)
10a			126.30	(q)	5a			129.15	(q)[f]
10b			65.26	(q)	6, 11-OH, 5'a			65.03	(q)[f]
11	5.03 s	10, 10'	81.89	(t)		6.08 br d (2.5)	10, 11'	82.92	(t)
12			75.11	(q)	5a			77.84	(q)
13	3.09 s	14	27.81	(p)		3.01 s	14	27.54	(p)
14	2.692 heptet (6.8)	13	32.24	(t)	15, 16	2.61 heptet (6.8)	13	32.69	(t)
15	1.45 d (6.8)		17.96	(p)	16	1.31 d (6.8)		18.35	(p)
16	1.45 d (6.8)		18.19	(p)	15	1.31 d (6.8)		18.35	(p)
11-OH	5.36 s	10', 11'				8.34 br d (2.5)			
1'			166.45	(q)	13'			168.33	(q)
3'			79.97	(q)	13', 15', 16'			80.84	(q)
4'			161.06	(q)				161.38	(q)
5'a	5.65 s	5a	77.02	(t)		7.11 s	5a	79.90	(t)
6'	4.47 s					*e*			
6'a			151.14	(q)				151.60	(q)
7'	6.51 d (7.8)		109.72	(t)		6.99 d (7.6)		108.69	(t)
8'	7.24 t (7.8)		129.88	(t)		7.09 t (7.6)		129.05	(t)
9'	6.95 t (7.8)		118.98	(t)		6.57 t (7.6)		117.24	(t)
10'	7.70 t (7.8)	11,11-OH,11'-OH	127.90	(t)		6.82 d (7.6)	11'-OH	130.93	(t)
10'a			123.82	(q)				125.08	(q)[f]
10'b			63.81	(q)				62.04	(q)[f]
11'	5.70 d (1.4)	5a, 11-OH	75.78	(t)		7.00 br d (3.5)	10, 11	75.96	(t)
12'			77.24	(q)	11'-OH			81.90	(q)
13'	3.08 s	14'	28.15	(p)		3.10 s	14'	27.96	(p)
14'	2.686 heptet (6.8)	13'	32.35	(t)	15', 16'	2.64 heptet (6.8)	13'	32.81	(t)
15'	1.41 d (6.8)		18.57	(p)	16'	1.33 (6.8)		18.12	(p)
16'	1.41 d (6.8)		18.57	(p)	15'	1.42 (6.8)		18.27	(p)
11'-OH	5.06 d (1.4)	10'				9.55 br d (3.5)	10'		

a ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (*J* /Hz) in parentheses. *b* Observed in the selected difference NOE experiment. *c* Letters, p, s, t and q , in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons. *d* Correlation from C to H observed in the long range ^{1}H-^{13}C COSY experiment. *e* Not detected. *f* Interchangeable.

The relative configuration of **4** was deduced from detailed analysis of NOE data of **4** and **8** in $CDCl_3$ (Tables 1 and 2). NOE correlations between 11-H and 12-SMe, and10-H and 11-H observed in the NOESY spectrum of **8** implied that 12-SMe, 11-H and the C-10a–C-10b bond are oriented *cis* to one another. Since the N-6–C-5a bond must be on the same side of the C-10a–C-10b bond, 5a-H and the C-10b–C-10'b bond should be *trans* to 11-H. In addition, NOEs between 11'-H and 11-H, and 11'-H and 10-H showed 11'-H to be oriented *cis* to the C-10'b–C-10b bond, while NOEs between 5'a-H and 16'-H, and 15'-H and 11'-H indicated that 5'a-H and the 3'-isopropyl group are on the same side of 11'-H. Because of the presence of sulfide bridges between C-3 and C-12, and C-3' and C-12' in **4**, the two S-Me groups on each of two

Table 2 ^{1}H and ^{13}C NMR data of derivative **8** in $CDCl_3$ and pyridine-d_5

position	$CDCl_3$				pyridine-d_5	
	δ ^{1}H[a]	NOEs (H)[b]	δ ^{13}C	LR ^{1}H–^{13}C correlation (C)[d]	δ ^{1}H	NOEs (H)[e]
1			166.02 (q)[c]			
3			79.13 (q)			
4			164.41 (q)			
5a	5.89 s (0.5)	5'a, 15	79.64 (t)	4, 10b, 10'b,	6.85 s	5'a, 15, 16
6	4.96 br s				*f*	
6a			150.93 (q)			
7	6.36 dd (7.8, 0.6)		108.96 (t)		6.64 dd (7.6, 0.9)	
8	7.15 td (7.8, 1.0)		129.71 (t)	6a, 10	7.30 td (7.6, 1.0)	
9	6.82 td (7.8, 0.6)		118.19 (t)		7.00 td (7.6, 0.9)	
10	7.76 dd (7.8, 1.0)	11, 12-SMe, 11'	125.89 (t)	6a, 8	8.21 dd (7.6, 1.0)	11, 12-SMe,11'
10a			128.39 (q)			
10b			62.35 (q)			
11	5.19 d (2.8)	10, 12-SMe, 11'	81.17 (t)	5a, 10a	5.69 d (6.7)	10, 12-SMe, 11'
12			72.49 (q)			
13	3.06 s	14, 3-SMe	29.83 (p)	1, 3	3.22 s	14, 15, 3-SMe
14	2.45 heptet (6.8)	13	37.49 (t)	3, 4, 15, 16	2.56 heptet (6.8)	13
15	1.02 d (6.8)	5a, 5'a	17.96 (p)	3, 14, 16	1.40 d (6.8)	5a, 13, 11-OH, 5'a
16	1.23 d (6.8)	5'a	17.86 (p)	3, 14, 15	1.45 d (6.8)	5a
11-OH	3.15 br d (2.8)				8.26 d (6.7)	15, 5'a, 11', 15'
3-SMe	2.21 s	13	13.66 (p)	3	2.09 s	13
12-SMe	1.98 s	10, 11	16.30 (p)	12	2.06 s	10, 11
1'			164.89 (q)			
3'			76.35 (q)			
4'			164.89 (q)			
5'a	5.87 s	5a, 15, 16, 16'	77.65 (t)	10b,6'a, 10'a, 11', 12', 10'b	6.62 s	5a, 15, 11-OH
6'	4.44 br s				*f*	
6'a			150.02 (q)			
7'	6.56 dd (7.8, 0.8)		108.58 (t)	9', 10'a	6.65 dd (7.6, 1.0)	
8'	7.06 td (7.8, 1.0)		129.37 (t)	6'a, 10'	7.02 td (7.6, 1.0)	
9'	6.32 td (7.8, 0.8)		117.85 (t)	7', 10', 10'a	6.45 td (7.6, 1.0)	
10'	5.82 dd (7.8, 1.0)		128.05 (t)	6'a, 8'	6.72 dd (7.6, 1.0)	12'-SMe
10'a			123.11 (q)			
10'b			60.33 (q)			
11'	5.50 d (11.8)	10, 11, 15'	78.79 (t)	10b, 1', 10'a, 10'b	6.47 s	10, 11, 11-OH, 16'
12'			73.37 (q)			
13'	3.06 s	14', 3-SMe	29.83 (p)	1', 3'	3.09 s	14', 15', 3'-SMe
14'	2.62 heptet (6.8)	13'	36.09 (t)	3', 4', 15', 16'	2.63 heptet (6.8)	13'
15'	1.03 d (6.8)	11'	17.86 (p)	3', 14', 16'	0.97 d (6.8)	11-OH, 13'
16'	1.08 d (6.8)	5'a	17.52 (p)	3', 14', 15'	1.19 d (6.8)	11'
11'-OH	3.14 d (11.8)				*f*	
3'-SMe	2.12 s	13'	13.82 (p)	3'	2.23 s	13'
12'-SMe	1.93 s		16.30 (p)	12'	2.20 s	10'

a ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (*J* /Hz) inparentheses. *b* Observed in the NOESY experiment *c* Letters, p, s, t and q , in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons. *d* Long range ^{1}H-^{13}C correlation from H to C observed in the HMBC experiment. *e* Observed in the selected difference NOE experiment. *f* Not detected.

dioxopiperazine rings in **8** should have *cis* orientations. Observation of an NOE between 5a-H and 15-H besides that between 5'a-H and 16'-H implied that both the dioxopiperazine rings in **8** exist in a chair conformation, with the isopropyl group in an axial arrangement. These findings and an NOE between 5a-H and 5'a-H observed in **4** allowed assignment of the relative configuration of **8** and consequently **4**. The relative configuration of C-3' and C-12' in **4** was different from that of leptosin A (**1**) – C (**3**),[5] G and H.[7]

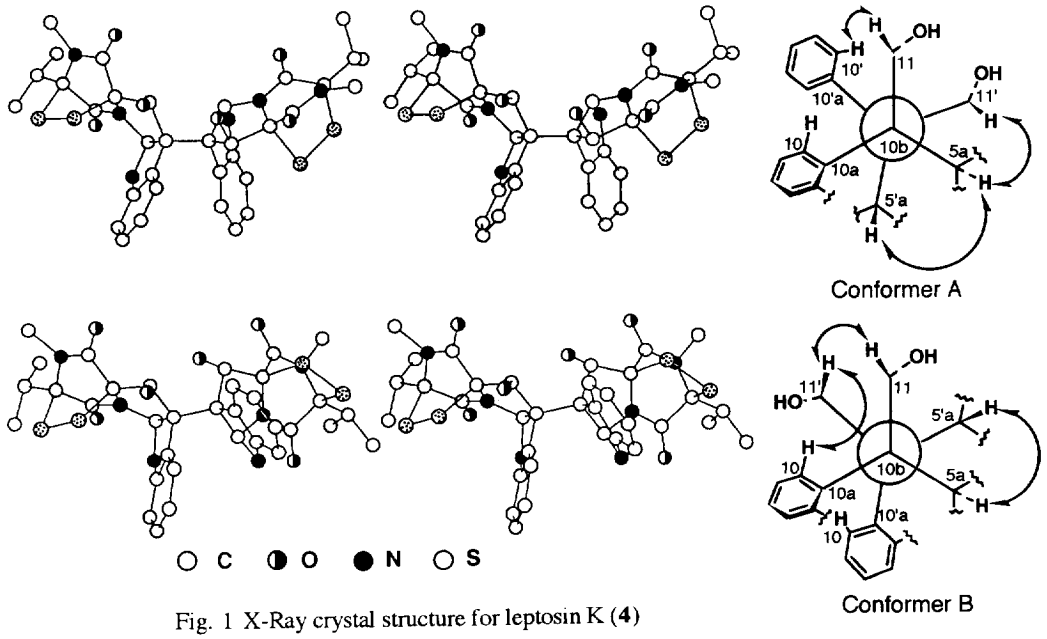


Fig. 1 X-Ray crystal structure for leptosin K (**4**)

In order to confirm the relative configuration of **4**, an X-ray structure analysis was carried out for its single crystal (obtained by recrystallization from AcOEt). Interestingly, the analysis demonstrated that four rotational isomers with the relative configuration mentioned above are incorporated in the single crystal of **4** in an equal ratio. These conformers are mainly formed by rotation of two monomeric subunits about the C-10b–C-10'b bond, and their rotational angles of the C-10a–C-10b and C-10'b–C-11' bonds were -166° , -165° , 74° and 70° , respectively. Since the rotational angles of two each of the four conformers are almost identical, they are respectively referred to as conformers A and B (Fig. 1) in the following discussion.

In NOE experiments in $CDCl_3$, **4** showed NOEs between 5a-H and 5'a-H, 5a-H and 11'-H, and 11-H and 10'-H, while **8** revealed NOEs between 5a-H and 5'a-H, 10-H and 11'-H, and 11-H and 11'-H as described above. Moreover, the NMR signals for 10-H and 10'-H in **4** appear at higher (δ 5.75) and lower (δ 7.70) fields, respectively, whereas their chemical shifts (δ 7.76, 10-H; δ 5.82, 10'-H) in **8** were in contrast with those of **4**. Their chemical shifts in **4** implied that 10-H on one benzene ring (C-6a– C-10a) lies above the plane of the other benzene ring (C-6'a–C-10'a), so as to be shielded by a field associated with the anisotropy of the latter benzene ring, and in such a conformation 10'-H on the benzene ring (C-6'a–C-10'a) should be situated in the position where it is deshielded by the other benzene ring (C-6a–C-10a). Based on the same consideration, their chemical shifts in **8** implied that 10'-H on the benzene ring (C-6'a–C-10'a) lies above the plane of the other benzene ring (C-6a–C-10a). This consideration showed **4** and **8** to exist singly in conformers of A and B types in $CDCl_3$, respectively. This experiment demonstrated that conformations in a series of dimeric dioxopiperazines in solution could be established by a combination of chemical shifts of 10-H and 10'-H, and NOE data between 5a-H, 10-H, 11-H, 10'-H and 11'-H. By application of this conformational approach, both **4** and **8** were shown to exist in a B type of conformer in pyridine-d_5 (Tables 1 and 2).

Table 3 ^{1}H and ^{13}C NMR data of derivative **7** in $CDCl_3$

position	Conformer A type			Conformer B type		
	$\delta^{1}H$[a]	$\delta^{13}C$	LR ^{1}H-^{13}C correlation (C)[c]	$\delta^{1}H$	$\delta^{13}C$	LR ^{1}H-^{13}C correlation (C)[c]
1		166.07 (q)[b]			165.76 (q)	
3		79.04 (q)			79.01 (q)	
4		165.42 (q)			164.66 (q)	
5a	5.93 s	78.95 (t)	6a, 10a, 12, 10'b	5.93 s	79.14 (t)	6a, 10a, 11, 12, 10'b
6	5.11 s			5.11 s		
6a		149.12 (q)			150.92 (q)	
7	6.51 d (7.8)	109.02 (t)	9,10a	6.39 d (7.8)	109.20 (t)	9, 10a
8	7.05 t (7.8)	129.87 (t)	6a, 10	7.16 t (7.8)	129.96 (t)	6a, 10
9	6.30 t (7.8)	118.65 (t)	7, 10a	6.81 t (7.8)	118.27 (t)	7, 10a
10	5.70 d (7.8)	124.10 (t)	6a, 8, 10b	7.69 d (7.8)	125.09 (t)	6a, 8
10a		127.07 (q)			128.22 (q)	
10b		63.86 (q)			62.40 (q)	
11	4.95 d (1.5)	80.84 (t)	5a, 10a	5.30 d (2.0)	81.30 (t)	5a, 10a, 10b
12		72.60 (q)			72.48 (q)	
13	3.11 s	30.06 (p)	1, 3	3.064 s	29.91 (p)	1, 3
14	2.50 heptet (6.8)	37.71 (t)	3, 4, 15, 16	2.46 heptet (6.8)	37.34 (t)	3, 4, 15, 16
15	1.11 d (6.8)	17.64 (p)	3, 14, 16	1.06 d (6.8)	17.77 (p)	3, 14, 16
16	1.27 d (6.8)	17.91 (p)	3, 14, 15	1.24 d (6.8)	17.91 (p)	3, 14, 15,
11-OH	4.66 br d (1.5)			3.64 d (2.0)		
3-SMe	2.16 s	13.96 (p)	3	2.13 s	13.96 (p)	3
12-SMe	2.09 s	16.42 (p)	12	2.11s	16.42 (p)	12
1'		166.16 (q)			167.73 (q)	
3'		80.31 (q)[e]			80.23 (q)[e]	
4'		160.84 (q)			161.04 (q)	
5'a	5.54 s	77.50 (t)	10b, 6'a, 10'a, 10'b, 12'	6.03 s	78.19 (t)	10b, 6'a, 10'a, 12'
6'	4.45 br s			4.45 br s		
6'a		151.55 (q)			149.67 (q)	
7'	6.51 d (7.8)	109.84 (t)	9', 10'a	6.61 d (7.8)	109.20 (t)	9', 10'a
8'	7.22 t (7.8)	129.87 (t)	6'a, 10'	7.07 t (7.8)	129.64 (t)	6'a, 10'
9'	6.91 t (7.8)	118.86 (t)	7', 10'a	6.37 t (7.8)	118.10 (t)	7', 10'a
10'	7.61 d (7.8)	127.75 (t)	6'a, 8'	5.92 d (7.8)	128.61 (t)	6'a, 8'
10'a		124.26 (q)			122.09 (q)	
10'b		64.92 (q)			62.10 (q)	
11'	5.44 br d (2.2)	75.45 (t)	10b, 1', 10'a	6.11 d (6.8)	76.35 (t)	10b, 1', 10'a
12'		77.50 (q)			79.58 (q)	
13'	3.056 s	28.17 (p)	1', 3'	3.04 s	28.17 (p)	1', 3'
14'	2.65 heptet (6.8)	32.45 (t)	3', 14', 15', 16'	2.70 heptet (6.8)	32.45 (t)	3', 14', 15', 16'
15'	1.39 d (6.8)	18.70 (p)	3', 14', 16'	1.45 d (6.8)	18.22 (p)	3', 14', 16'
16'	1.45 d (6.8)	18.70 (p)	3', 14', 15'	1.45 d (6.8)	18.30 (p)	3', 14', 15'
11'-OH	4.73 d (2.2)			3.41 d (6.8)		

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

Compounds **7** derived by treatment of **4** with $NaBH_4$ and MeI had the molecular formula $C_{36}H_{42}N_6O_6S_4$, agreeing with the conversion of one disulfide bridge of **4** into two S-Me groups, as deduced from an MH^+ peak of FABMS. Its fragmentation pattern of FABMS, and HPLC and TLC analyses secured purity of this compound; however, the ^{1}H and ^{13}C NMR spectra of **7** in $CDCl_3$ were very complex and suggested it existed in a 1 : 1 mixture of two isomers (Table 3). A detailed ^{1}H and ^{13}C NMR spectral analysis of **7** by HMBC and NOESY experiments led to the assignment of signals in the two isomers, and observation of an NOE between 11-H and 12-SMe in the two isomers (Table 4) revealed that 12-SMe is oriented *cis* to 11-

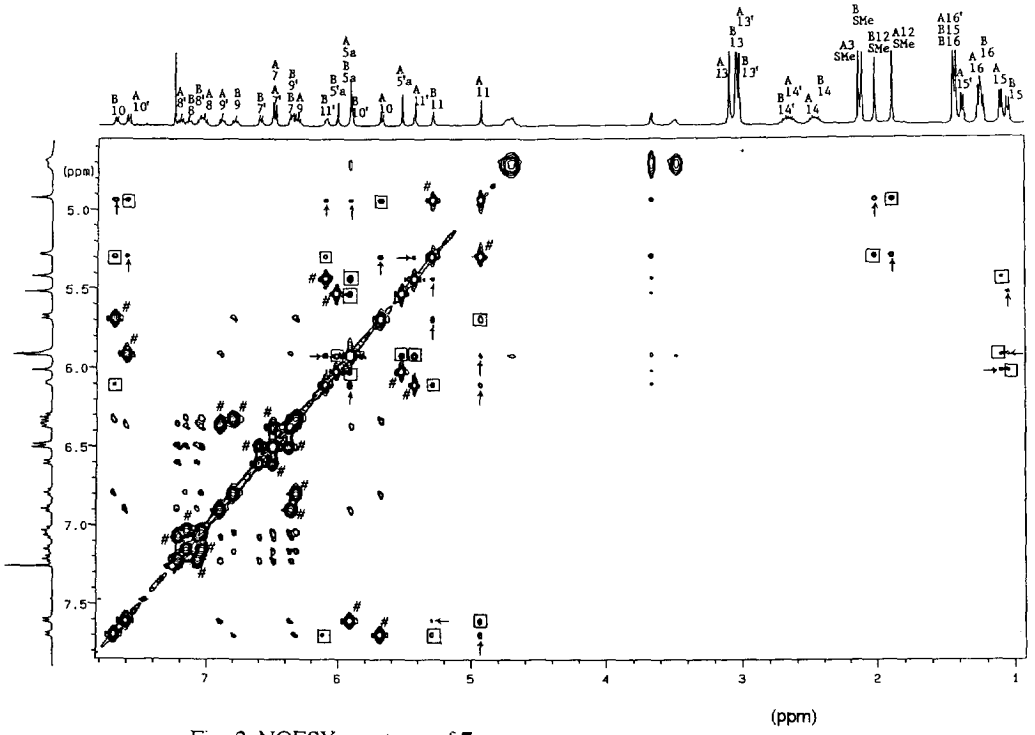


Fig. 2 NOESY spectrum of **7**

A : conformer A type; B: conformer B type. □ : part of nomal NOESY cross peaks; # : exchange cross peaks; ↑ : exchange-mediated NOESY cross peaks

H in both isomers, implying that they are not positional isomers of S-Me groups, but conformational ones with two S-Me groups at C-3 and C-12 and a disulfide bridge at C-3' and C-12'.

It is interesting that the phase-sensitive NOESY spectrum of **7** (Fig. 2) revealed the appearance of some intense additional cross peaks with minus signs besides normal cross peaks with plus signs. The cross peaks are assumed to arise due to a slow exchange between distinct conformations and hence may be referred to as conformational exchange cross peaks.[8] They are observed between equivalent protons of exchangeable distinct conformations as observed between interchangeable protons such as OH and NH in NOESY spectra. In addition, the NOESY spectrum exhibited some conformational exchange-mediated NOESY cross peaks with plus signs. The cross peaks indicate NOE correlations between protons of distinct conformations, arising from a transfer of NOE due to a conformational exchange, though the normal NOESY cross peaks are observed between protons in the same conformation. The NOESY spectral feature (Table 4) of **7** described above aupported the hypothesis that **7** exists in a mixture of two conformers, with slowly exchange occurring in $CDCl_3$. One of the two conformers exhibited the normal NOESY cross peaks between 11-H and 10-H, 11-H and 10'-H, 11'-H and 5a-H, and 5a-H and 5'a-H, and the lowfield-shifted signal (δ 7.61) for 10'-H (Tables 3 and 4), implying it to be an A type of conformer. The other exhibited the normal NOESY cross peaks between 10-H and 11-H, 11-H and 11'-H, and 5a-H and 5'a-H, and the lowfield-shifted signal (δ 7.69) for 10-H (Tables 3 and 4), implying it to be a B type of conformer. The lowfield-shifted signal (δ 8.18) for

Table 4 NOESY data of derivative **7** in $CDCl_3$

H	Normal cross peaks (H)		Exchange-mediated cross peaks (H)		Exchange cross peaks (H)	
	conformer A[a]	conformer B[b]	conformer A	conformer B	conformer A	conformer B
5a	15, 5'a, 11'	15, 5'a	B- 11[c]		B-5a	A-5a
7					B-7	A-7
8					B-8	A-8
9					B-9	A-9
10	11	11, 11'	B- 11	A- 11[d]	B-10	A-10
11	10, 10', 12-SMe	10, 11', 12-SMe	B- 10,10', 11', 12SMe	A- 10, 10', 11', 12SMe	B-11	A-11
13	14, 15, 16, 3-SMe	14, 15, 16, 3-SMe	B- 14, 15, 16	A- 14, 15, 16		
14	13	13	B- 13	A- 13		
15	5a, 13, 11'	5a, 13, 5'a	B- 13, 5'a	A- 13, 5'a		
16	13	13	B- 13	A- 13		
3-SMe	13	13	B- 13	A- 13		
12-SMe	11	11	B- 11	A- 11	B-12SMe	A-12-SMe
5'a	5a	5a, 15	B- 15	A- 15	B-5'a	A-5'a
7'					B-7'	A-7'
8'					B-8'	A-8'
9'					B-9'	A-9'
10'	11		B- 11	A- 11	B-10'	A-10'
11'	5a, 15	11, 10	B- 11	A- 5a, 11	B-11'	A-11'
13'	14', 15', 16'	14', 15', 16'	B-14', 15', 16'	A- 14', 15', 16'		
14'	13'	13'	B- 13'	A- 13'		
15'	13'	13'	B- 13'	A- 13'		
16'	13'	13'	B- 13'	A- 13'		

a Conformer A type. *b* Conformer B type. *c* Positions of protons in the conformer B type. *d* Positions of protons in the conformer A type.

10-H and NOE data (Table 7) of **7** observed in pyridine-d_5 instead of $CDCl_3$ indicated that **7** exists only in a B type of conformer in the solution. The conformational exchange and exchange-mediated NOESY cross peaks have previously been observed in phase-sensitive 2D NOESY spectra of large molecules such as DNA oligomers[8,9a] and proteins,[9b] but their observation for the lower molecule as described above is a frequent instance.

Leptosin K_2 (**6**) was assigned the molecular formula $C_{34}H_{36}N_6O_6S_6$ as deduced from an MH^+ peak in HRFABMS. This compound exhibited complex ^1H and ^{13}C NMR spectra (Table 5) like **7** in $CDCl_3$, suggesting that **6** has functionalities similar to those of **4**, but that it existed in a 1 : 1 mixture of two conformers in $CDCl_3$. This was supported by the NOESY spectrum (Table 6) of **6** as described below. Assignments of the ^1H and ^{13}C NMR signals for each of two conformers by ^1H–^1H and ^1H –^{13}C COSY and HMBC correlations (Table 5) revealed that chemical shifts of the ^1H and ^{13}C signals for one monomeric subunit (C-1'– C-16') in both conformers were almost coincident with those of **7** and **4**, and hence the monomeric subunit of **6** is the same as that of **4** and **7** with a disulfide bridge. Treatment of **6** with $NaBH_4$ and MeI afforded methylsulfanyl derivatives **7** and **8**. Formation of **7** from **6** and the molecular formula of **6** showed the other monomeric subunit (C-1– C-16) to have a tetrasulfide bridge. This was supported by the appearance of FABMS fragments at *m/z* 556 (h^+), 493 (cH^+), 440 (g^+), 428 ($[c-2S]^+$ or $[h-4S]^+$) and 376 (b^+). This evidence led to structure **6** for leptosin K_2, with the same configuration as **4**.

The general features of the NOESY spectrum of **6** closely resembled those of **7**, exhibiting exchange, exchange-mediated and normal NOESY cross peaks, except for cross peaks about the S-Me groups. Analysis of the normal NOESY cross peaks (Table 6) revealed that **6** exists in a 1 : 1 mixture of conformers

Table 5 ^{1}H and ^{13}C NMR data of Leptosin K_2 (**6**) in $CDCl_3$ at 27°C

	Conformer A type			Conformer B type		
position	$\delta^{1}H$[a]	δ ^{13}C	LR ^{1}H-^{13}C correlation (C)[c]	$\delta^{1}H$	δ ^{13}C	LR ^{1}H-^{13}C correlation (C)[c]
1		167.73 (q)[b]			167.73 (q)	
3		81.85 (q)			81.85 (q)	
4		167.73 (q)[d]			167.89 (q)[d]	
5a	6.05 s	79.30 (t)	6a, 10a, 12, 10'b	6.08 s	79.69 (t)	6a, 10a, 12, 10'b
6	5.17 s			5.17 s		
6a		148.42 (q)			150.06 (q)	
7	6.43 d (7.8)	108.94 (t)	9	6.25 d (7.8)	108.94 (t)	9, 10a
8	6.98 t (7.8)	130.41 (t)	6a, 10	7.06 t (7.8)	130.41 (t)	6a, 10
9	6.33 t (7.8)	119.73 (t)	7, 10a	6.71 t (7.8)	119.10 (t)	7, 10a
10	5.64 d (7.8)	125.69 (t)	6a	7.62 d (7.8)	126.80 (t)	6a
10a		126.80 (q)			127.80 (q)	
10b		63.84 (q)			62.50 (q)	
11	4.93 s	83.36 (t)		5.38 s	83.79 (t)	
12		79.99 (q)			80.36 (q)	
13	3.04 s	30.20 (p)		3.05 s	30.45 (p)	
14	2.69 heptet (6.8)	36.05 (t)	3	2.69 heptet (6.8)	36.28 (t)	3
15	1.15 d (6.8)	18.32 (p)	3	1.15 d (6.8)	18.32 (p)	3
16	1.43 d (6.8)	18.32 (p)	3	1.43 d (6.8)	18.32 (p)	3
11-OH	4.29 s			4.98 s		
1'		166.32 (q)			167.73 (q)	
3'		80.36 (q)			80.36 (q)	
4'		161.11 (q)			161.11 (q)	
5'a	5.55 s	77.56 (t)	10b, 6'a, 12'	6.07 s	78.26 (t)	10b, 6'a, 10'a,12'
6'	4.47 s			4.47 s		
6'a		151.43 (q)			149.61 (q)	
7'	6.50 d (7.8)	109.85 (t)	9', 10'a	6.63 d (7.8)	109.24 (t)	9', 10'a
8'	7.22 t (7.8)	130.08 (t)	6'a, 10'	7.08 t (7.8)	129.67 (t)	6'a, 10'
9'	6.91 t (7.8)	119.10 (t)	7', 10'a	6.37 t (7.8)	118.27 (t)	7', 10'a
10'	7.61 d (7.8)	127.80 (t)	6'a, 8'	6.02 d (7.8)	128.89 (t)	6'a, 8'
10'a		123.92 (q)			122.25 (q)	
10'b		64.55 (q)			61.59 (q)	
11'	5.50 s	75.70 (t)	10b, 1', 10'a	6.27 d (6.5)	76.09 (t)	10b, 10'a
12'		77.14 (q)			79.99 (q)	
13'	3.06 s	28.33 (p)	1', 3'	3.08 s	28.33 (p)	1'
14'	2.67 heptet (6.8)	32.49 (t)	3'	2.67 heptet (6.8)	32.49 (t)	3'
15'	1.41 d (6.8)	18.77 (p)	3'	1.46 d (6.8)	18.48 (p)	3'
16'	1.46 d (6.8)	18.77 (p)	3'	1.46 d (6.8)	18.48 (p)	3'
11'-OH	4.68 s			4.06 s		

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

of A and B types slowly exchanging in $CDCl_3$. The ^{1}H NMR signals of **6** became broad and individual protons were not distinguished when measured in $CDCl_3$ at 60°C, whereas when measured at 0°C they appeared as sharper and clearer signals than at 27°C. When measured in pyridine-d_5 at 27°C, the ^{1}H and ^{13}C NMR spectra of **6** were found it to exist only in a single isomer, which was deduced as a B type from NOEs between 11'-H and 11-H, 11'-H and 10-H, and 5a-H and 5'a-H and appearance of the lowfield-shifted signal (δ 8.04) for 10-H (Table 7).

The molecular formula of leptosin K_1 (**5**) was established as $C_{34}H_{36}N_6O_6S_5$ by HRFABMS. Since the ^{1}H NMR spectrum of **5** in $CDCl_3$ at 27°C appeared as broad signals so that only isopropyl and N-methyl

Table 6 NOESY data of leptosin K_2 (**6**) in $CDCl_3$ at 27°C

	Normal cross peaks (H)		Exchange-mediated cross peaks (H)		Exchange cross peaks (H)	
H	conformer A[a]	conformer B[b]	conformer A	conformer B	conformer A	conformer B
5a	5'a, 11'	5'a	B-11[c]			
6					B-6	A-6
7					B-7	A-7
8					B-8	A-8
9					B-9	A-9
10	11	11, 11', 11'-OH	B-11	A-11[d]	B-10	A-10
11	10, 10'	10, 11'	B-10, 10', 11'	A-10, 10', 11'	B-11	A-11
13	14	14				
14	13	13				
11-OH	10', 11'	11'			B-11-OH	A-11-OH
5'a	5a	5a				
6'					B-6'	A-6'
7'					B-7'	A-7'
8'					B-8'	A-8'
9'					B-9'	A-9'
10'	11,11-OH,11'-OH	11'-OH	B-11, 11'OH	A-11, 11'OH	B-10'	A-10'
11'	5a, 11-OH	10, 11, 11-OH	B-11	A-5a, 11	B-11'	A-11'
13'	14'	14'				
14'	13'	13'				
11'-OH	10'	10, 10'	B-10'	A-10'	B-11'-OH	A-11'-OH

a Conformer A type. *b* Conformer B type. *c* Positions of protons in the conformer B type. *d* Positions of protons in the conformer A type

groups could be assigned. The ^1H NMR spectrum resulting from measurement at 0°C (Table 8) showed sharper and clearer signals, and the NOEs (Table 8) demonstrated that **5** exists in a 3 : 2 mixture of conformers of A and B types in $CDCl_3$. Chemical shifts of the ^1H NMR signals for one monomeric subunit (C-1'– C-16') of both conformers were almost coincident with those of **4**, **6** and **7**, implying that the monomeric subunit of **5** is the same as that of **4**, **6** and **7** with a disulfide. Treatment of **5** with $NaBH_4$ and MeI gave **7** as well as **8**. Formation of **7** from **5** and the molecular formula of **5** showed the other monomeric subunit (C-1–C-16) to have a trisulfide bridge. This was supported by FABMS fragments at *m/z* 524 (f^+), 493 (cH^+), 428 ($[f-3S]^+$ or $[c-2S]^+$) and 376 (b^+). In addition, **5** was derived from **6** by treatment with triphenylphosphine. The above evidence allowed assignment of structure **5** with the same configuration as that of **4** and **6** for leptosin K_1. Incidentally, the ^1H and ^{13}C NMR signals of **5** in pyridine-d_5 showed it to exist in a single conformer, which was deduced as a B type from NOEs and the chemical shift for 10-H (Table 8). Solution conformations of **4**–**8** described above are summarized in Table 9.

The absolute configuration of leptosin C (**3**) was previously determined by comparison of a 271 nm band due to an S→CO charge-transfer transition in the circular dichroism (CD) spectrum of **3** with that of di-O-acetylchaetocin (**9**).[5] The CD spectrum of **4** (Fig. 3) exhibited a positive band at 264 nm, but it seems unreasonable that the absolute configuration of **4** be deduced from this CD band because the configurations for the two disulfide bridges in the dioxopiperazine rings of **4** are reverse to each other. Leptosin B (**2**) was treated with triphenylphosphine at room temperature to afford **10** as reported previously.[5] Treatment of **5** with triphenylphosphine at 60°C gave, besides **4**, compound **11** in which one monomeric subunit of **5** was replaced by a 3-substituted indole moiety. Comparison of the FABMS, ^1H-NMR and NOE data of **11** with those of **10** revealed that **10** and **11** are isomers with different relative configurations of C-11. NOE between H-10 and H-11 was observed in **10**, but not in **11**, implying that the dioxopiperazine ring in **11** came from the monomeric subunit (C-1'–C-16') of **5**. There was a negative band at 266 nm due to an S→CO charge-

Table 7 ^{1}H and ^{13}C NMR data of derivatives **6** and **7** in pyridine-d_5

	6				**7**	
porsition	δ $^{1}H^{a}$	NOEs (H)^{b}	δ ^{13}C	LR ^{1}H–^{13}C correlation (H)^{d}	δ ^{1}H	NOEs (H)^{b}
1			167.61 (q)^{c}			
3			82.57 (q)	13, 15, 16		
4			166.88 (q)			
5a	6.85 s	5'a	79.85 (t)		6.77 s	5'a
6	7.76 s				7.81 s	
6a			153.06 (q)	5a, 8, 10		
7	6.60 d (7.6)		108.80 (t)	9	6.69 d (7.6)	
8	7.24 t (7.6)		130.24 (t)	10	7.35 t (7.6)	
9	6.889 t (7.6)		117.32 (t)	7	7.00 t (7.6)	
10	8.04 d (7.6)	11, 11'	126.99 (t)	8	8.18 d (7.6)	11, 12-SMe, 11'
10a			130.02 (q)	7, 9		
10b			63.87 (q)	5'a		
11	5.83 d (5.5)	10, 11'	84.88 (t)		5.84 d (5.3)	10, 12-SMe, 11'
12			81.48 (q)	5a		
13	3.06 s	14	29.75 (p)		3.13 s	14, 15, 3-SMe
14	2.61 heptet (6.8)	13	35.97 (t)	16	2.44 heptet (6.8)	13
15	1.09 d (6.8)		18.17 (p)		1.27 d (6.8)	13, 5'a
16	1.38 d (6.8)		18.50 (p)		1.35 d (6.8)	
11-OH	9.54 d (5.5)				8.70 d (5.3)	5'a, 11'
3-SMe					2.08 s	13
12-SMe					2.07 s	10, 11
1'			168.32 (q)	11', 13'		
3'			80.92 (q)	13', 15', 16'		
4'			161.29 (q)	14'		
5'a	6.95 s	5a	79.33 (t)		6.96 s	5a, 11-OH, 15
6'	*e*				*e*	
6'a			151.65 (q)	5'a, 8', 10'		
7'	6.71 dd (7.6, 0.5)		108.61 (t)	9'	6.67 d (7.6)	
8'	7.07 td (7.6, 1.0)		129.18 (t)	10'	7.05 t (7.6)	
9'	6.57 td (7.6, 0.5)		117.12 (t)	7'	6.56 t (7.6)	
10'	6.892 dd (7.6, 1.0)		131.05 (t)	8'	6.85 d (7.6)	
10'a			125.20 (q)	7', 9', 11'		
10'b			62.86 (q)	5a		
11'	6.97 br s	10, 11	76.18 (t)		7.00 s	10, 11, 11-OH
12'			81.91 (q)	5'a		
13'	3.07 s	14'	27.93 (p)		3.08 s	15', 14'
14'	2.62 heptet (6.8)	13'	32.79 (t)	16'	2.63 heptet (6.8)	13'
15'	1.27 d (6.8)		18.50 (p)	16'	1.31 d (6.8)	13'
16'	1.43 d (6.8)		18.29 (p)	15'	1.44 d (6.8)	
11'-OH	*e*				9.43 br s	

a ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (*J* /Hz) in parentheses. *b* Observed in the selected difference NOE experiment. *c* Letters, p, s, t and q , in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons. *d* Correlation from C to H observed in the long range ^{1}H-^{13}C COSY experiment. *e* Not detected.

transfer transition in the CD spectrum (Fig. 3) of **10**, which has the *S* configuration at both C-3 and C-12. On the other hand, **11** had a positive band at 268 nm, implying that the asymmeric centers at both C-3 and C-12 have the *R* configuration. In the ^{1}H NMR spectra, the 4'-H signal of **11** was found shifted upfield by 0.41ppm, relative to **10**. This large upfield shift suggests that the relative configuration of the hydroxy group at C-11 exerts a favorable influence upon rotation about C-10b–C-3' bond in both compounds. It is assumed that the differences in conformation of both compounds might effect some differences in strength between their corresponding CD bands. An opposite sign of the charge-transfer bands at *ca.* 266 nm was also observed in

Table 8 ^{1}H and ^{13}C NMR spectral data of leptosin K_1 (**5**) in $CDCl_3$[a] and pyridine-d_5

	$CDCl_3$				pyridine-d_5		
	conformer A		conformer B				
position	δ ^{1}H[b]	NOEs (H)[c]	δ ^{1}H	NOEs (H)[c]	δ ^{1}H	NOEs (H)[c]	δ ^{13}C
1							169.23 (q)[d]
3							81.41 (q)
4							164.14 (q)
5a	5.90 s	5'a, 11'	5.82 s	5'a	6.59 s	5'a	78.77 (t)
6	5.24 br s[f]		5.52 br s[f]		*e*		
6a							155.13 (q)
7	6.46 d (7.8)		6.37 d (7.8)		6.69 d (7.6)		109.81 (t)
8	7.14 t (7.8)		7.08 t (7.8)		7.34 t (7.6)		131.01 (t)
9	6.40 t (7.8)		6.62 t (7.8)		6.96 t (7.6)		117.21 (t)
10	5.65 d (7.8)	11	7.56 d (7.8)	11, 11'	8.05 d (7.6)	11, 11'	128.12 (t)
10a							126.24 (q)
10b							61.81 (q)[g]
11	5.00 s	10, 10'	5.61 s	10, 11'	6.12 s	10, 11'	85.06 (t)
12							85.23 (q)
13	3.25 s	14	3.20 s	14	3.22 s	14	27.74 (p)
14	2.50 heptet (6.8)	13	2.50 heptet (6.8)	13	2.38 heptet (6.8)	13	35.35 (t)
15	1.23 d (6.8)		1.23 d (6.8)		0.83 d (6.8)		18.25 (p)
16	1.46 d (6.8)		1.46 d (6.8)		1.38 d (6.8)		18.25 (p)
11-OH	5.26 s	10', 11'	4.72 s		*e*		
1'							168.09 (q)
3'							80.88 (q)
4'							161.36 (q)
5'a	5.66 s	5a	6.28 s	5a	7.13 s	5a	79.70 (t)
6'	4.54 br s		4.72 br s		*e*		
6'a							151.63 (q)
7'	6.50 d (7.8)		6.69 d (7.8)		6.72 d (7.6)		108.74 (t)
8'	7.24 t (7.8)		7.14 t (7.8)		7.10 t (7.6)		129.05 (t)
9'	6.91 t (7.8)		6.38 t (7.8)		6.57 t (7.6)		117.30 (t)
10'	7.58 d (7.8)	11, 11-OH, 11'-OH	5.97 d (7.8)		6.80 d (7.6)		130.73 (t)
10'a							125.51 (q)
10'b							63.18 (q)[g]
11'	5.66 s	5a, 11-OH	6.32 s	10, 11	6.82 s	10,11	75.97 (t)
12'							81.89 (q)
13'	3.08 s	14'	3.06 s	14'	3.08 s	14'	27.83 (p)
14'	2.68 heptet (6.8)	13'	2.68 heptet (6.8)	13'	2.66 heptet (6.8)	13'	32.75 (t)
15'	1.41 d (6.8)		1.41 d (6.8)		1.32 d (6.8)		18.41 (p)
16'	1.46 d (6.8)		1.46 d (6.8)		1.43 d (6.8)		18.25 (p)
11'-OH	5.18 s	10'	4.54 br s[f]		*e*		

a Measured at 0°C in $CDCl_3$. *b* ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (*J* /Hz) in parentheses. *c* Observed in the selected difference NOE experiment. Exchange and exchange-mediated NOE data are omitted. *d* Letters, p, s, t and q , in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons. *e* Not detected. *f*, *g* Interchangeable.

bis(methylsulfanyl) derivatives **7**, and **12** with the *S* configuration at both C-3 and C-12,[5] implying that the asymmetric centers at C-3' and C-12' in **7** have the *R* configuration. Based on the above summarized evidence, the absolute stereostructures of leptosin K_1 and, consequently, leptosins K and K_2 are represented as **5**, **4** and **6**, repectively.

The cytotoxic activities of leptosin K (**4**), K_1 (**5**) and K_2 (**6**) were examined in the P388 lymphocytic leukemia test system in cell culture, according to the method reported previously.[10] As shown in Table 10, all the compounds tested exhibited potent cytotoxic activity.

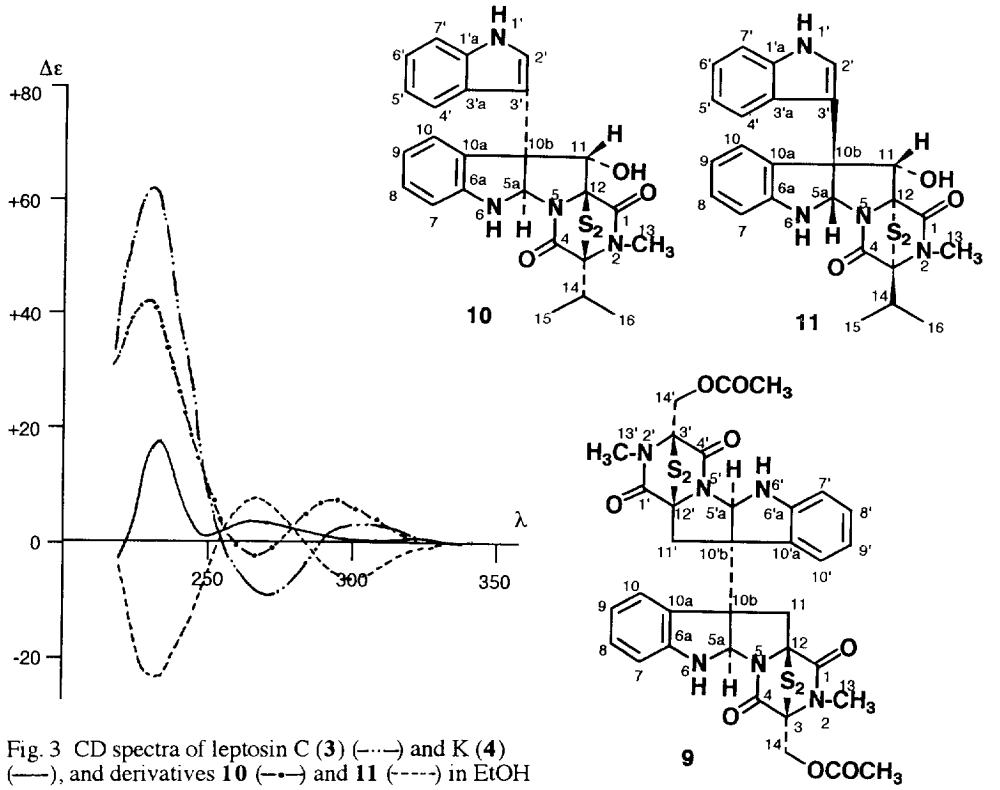


Fig. 3 CD spectra of leptosin C (**3**) (–···–) and K (**4**) (——), and derivatives **10** (–•–) and **11** (-----) in EtOH

Table 9 Conformers (A or B type) of leptosins and derivatives in solution

Solvent	**4**	**5**	**6**	**7**	**8**
$CDCl_3$	A	A /B (3 / 2)	A /B (1 /1)	A /B (1 /1)	B
pyridine-d_5	B	B	B	B	B

Table 10 Cytotoxicity of leptosins K (**4**)–K_2 (**6**) against tumour cells

Compound	Cell line P 388 (ED_{50} μg/ml)
Leptosin K (**4**)	3.8×10^{-3}
Leptosin K_1 (**5**)	2.2×10^{-3}
Leptosin K_2 (**6**)	2.1×10^{-3}
Mitomycin C (standard)	4.0×10^{-2}

Experimental

General Procedures. — M.p.s were obtained on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR spectrometer 1720X. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. CD spectra were recorded on a JASCO J-500A spectrometer. NMR spectra were recorded at 27°C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^1H and ^{13}C, respectively, with tetramethylsilane (TMS) as an internal reference. ^1H–^1H and ^1H–^{13}C COSY spectra including both one-bond and long range correlations were recorded on a Varian XL-300 spectrometer, and HMBC and NOESY spectra on a Varian

UNITY-400 spectrometer with the usual parameters except that the NOESY spectrum for **6** was recorded on a Bruker ARX-500 spectrometer.

FABMS was determined using a VG ZAB-SE mass spectrometer and a JEOL JMS-HX 100/110A mass spectrometer in 3-nitorobenzyl alcohol matrix. Liquid chromatography over silica gel (mesh 230-400) was performed in a medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-SIL or -ODS (25 cm x 20 mm i. d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent CH_2Cl_2–MeOH (97 : 3 and 9 : 1), and compounds were viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Isolation of Metabolites. — As reported previously,[5] a strain of *Leptosphaeria* sp. isolated from the marine alga *Sargassum tortile* C. AGAROH (Sargassaceae) was cultured in a liquid medium (20 l) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5 at 27°C. The CH_2Cl_2–MeOH (1:1) soluble fraction (21.5 g) of the MeOH extract for the mycelium was successively chromatographed on Sephadex LH-20 (CH_2Cl_2–MeOH, 1 : 1) and silica gel (hexane–CH_2Cl_2 gradient). The hexane–CH_2Cl_2 (6 : 4) and (4 : 6) eluates were collected as 2 fractions [Fr. 2 (78 mg) and Fr. 3 (139 mg)] and one fraction [Fr. 5 (965 mg)], respectively. Fr. 2 was purified by HPLC (SIL) using CH_2Cl_2 as the eluent to afford **4** (14.6 mg). Fr. 3 and Fr. 5 afforded **5** (28.8 mg) and **6** (29 mg), respectively, after purification by HPLC (SIL) using acetone– CH_2Cl_2 (2 : 98) as the eluent.

Leptosin K (**4**). This was obtained as colorless prisms, m.p. 222–224°C (from AcOEt), $[\alpha]_D$ +76.7 (*c* 0.37 in $CHCl_3$); λ_{max} (EtOH)/nm 206 (log ε 4.78), 240 (4.28) and 302 (3.78); υ_{max} (KBr)/cm^{-1} 3408 (OH, NH), 1691, 1665 (CON), 1608 and 1595 (Ar. C-C); *m/z* (FAB) 753 (15 %, MH^+), 688 (11, M^+–2S), 670 (1.5, M^+–2S–H_2O), 493 (1.3 , cH^+ or dH^+), 428 (11, c^+ or d^+–2S), 410 (2, c^+ or d^+ –2S–H_2O), 376 (2 , a^+ or b^+), 312 (9 , a^+or b^+–2S), 232 (100, [bi-indol-3-yl]$^+$) and 197 (48, cH^+ or dH^+–2S–232) (Found: MH^+, 753.1664. $C_{34}H_{37}N_6O_6S_4$ requires *M* H^+, 753.1658); CD λ (*c* 2.77 x 10^{-5} M in EtOH)/nm 234 (Δε +17.8), 249 (+1.1), 264 (+4.8), 306 (+1.3) and 319 (+1.4). ^1H and ^{13}C NMR data are listed in Table 1.

Leptosin K_1 (**5**). This was obtained as a pale yellow powder, m.p. 209–212 °C, $[\alpha]_D$ + 88.9 (*c* 0.32 in $CHCl_3$); λ_{max} (EtOH)/nm 207 (log ε 4.71), 237 (4.29) and 300 (3.74); υ_{max} (KBr)/cm^{-1} 3414 (OH, NH), 1689, 1658 (CON), 1608 and1595 (Ar. C-C); *m/z* (FAB) 785 (3 %, MH^+), 721 (5, MH^+–2S), 703 (0.9, MH^+–2S– H_2O), 689 (0.3, MH^+–3S), 671 (0.4, MH^+–3S–H_2O), 624 (0.2 , M^+–5S), 524 (3, f^+), 493 (0.7, cH^+), 428 (5, c^+–2S or f^+–3S), 376 (2, b^+), 312 (16, e^+–3S), 232 (100 , [bi-indol-3-yl]$^+$) and 197 (58 , cH^+–2S–232) (Found: MH^+, 785.1380. $C_{34}H_{37}N_6O_6S_5$ requires *M* H^+, 785.1378); CD λ (*c* 3.02 x 10^{-5} M in EtOH)/nm 238 (Δε +4.5), 242 (+4.1), 264 (+18.8), 300 (-1.3) and 325 (+4.0). ^1H and ^{13}C NMR data are listed in Table 8 .

Leptosin K_2 (**6**). This was obtained as a pale yellow powder, m.p. 214–216 °C, $[\alpha]_D$ + 482.8 (*c* 0.44 in $CHCl_3$); λ_{max} (EtOH)/nm 205 (log ε 4.74), 242 (4.15) and 296 (3.64); υ_{max} (KBr)/cm^{-1} 3375 (OH, NH), 1693 and 1664 (CON), 1608, 1591 (Ar. C-C); *m/z* (FAB) 817 (63 %, MH^+), 753 (81, MH^+–2S), 752 (100, M^+–2S), 689 (19, MH^+–4S), 671 (21 , MH^+–4S–H_2O), 624 (16, M^+–6S), 556 (3, h^+), 493 (15, cH^+), 440 (3, g^+), 428 (91, c^+–2S or h^+–4S), 411 (18, cH^+–2S–H_2O), 410 (63, h^+–4S–H_2O), 376 (2, b^+) and 232 (83, [bi-indol-3-yl]$^+$) (Found: MH^+, 817.1102. $C_{34}H_{37}N_6O_6S_6$ requires *M* H^+, 817.1099); CD λ (*c* 1.81 x 10^{-5} M in EtOH)/nm 234 (Δε +14.6), 248 (+1.4), 266 (+12.3), 300 (+4.2), 312 (+2.9) and 345 (-0.84). ^1H and ^{13}C NMR data are listed in Tables 5 and 7.

Formation of the Bis (methylsulfany) and Tetrakis (methylsulfanyl) Derivatives **7** *and* **8** *from Leptosins K* (**4**), K_1 (**5**) *and* K_2 (**6**) *each.* — **4** (11.6 mg) was dissolved in a solution (0.25 ml) of pyridine and MeOH (5 : 8). MeI (1 ml) and $NaBH_4$ (4 mg) were added, and the mixture was stirred for 20 min at room temperature. The reaction mixture was then diluted with water and extracted with diethyl ether. The solvent was evaporated off under reduced pressure, and the residue was chromatographed on a silica gel column with a CH_2Cl_2 as the eluent. The eluate was purified by HPLC (ODS) using MeOH–H_2O (4 : 1) to afford **7** (4.4 mg) and **8** (2.2 mg). **7** was obtained as a pale yellow oil; $[\alpha]_D$ -62.5 (*c* 0.08 in $CHCl_3$); λ_{max} (EtOH)/nm 214 (log ε 4.58), 240 (4.20), 302 (3.73); υ_{max} (KBr)/cm^{-1} 3546 (OH, NH), 1680, 1661 (CON),1609 and 1593 (Ar. C-C); *m/z* (FAB) 783 (MH^+), 789 ($[M + Li]^+$); CD λ (*c* 5.12 x10^{-5} M in EtOH)/nm 221 (Δε -30.2), 242 (-21.3), 266 (+7.7), 295 (-0.59), 316 (+1.2) and 335 (-0.30). ^1H and ^{13}C NMR data are listed in Tables 3 and 7. **8** was obtained as a pale yellow oil, $[\alpha]_D$ -59.3 (*c* 0.27 in $CHCl_3$); λ_{max} (EtOH)/nm 212 (log ε 4.74), 240 (4.19) and 302 (3.68); υ_{max} (KBr)/cm^{-1} 3443 (OH, NH), 1675, 1658 (CON),1609 and 1594 (Ar. C-C); *m/z* (FAB) 812 (M^+), 819 ($[M + Li]^+$); CD λ (*c* 6.65 x10^{-5} M in EtOH)/nm 227 (Δε -8.9), 242 (-8.0), 269 (+2.7), 296 (+0.46) and 314 (+0.91). ^1H and ^{13}C NMR data are listed in Table 2.

The same reaction with **5** (20 mg) and **6** (8.2 mg) gave **7** (7.2 mg and 2.4 mg, respectively) and **8** (4.3 mg and 1.2 mg, respectively).

Formation of Leptosin K_1 (**5**) *from Leptosin* K_2 (**6**). — Triphenylphosphine (6 mg) was added to a $CHCl_3$ solution (2 ml) of **6** (12 mg), and the reaction mixture was left to stand at room temperature for 1.5 hr. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using CH_2Cl_2 to afford **5** (5.9 mg) , which was identified by IR, ^1H NMR, CD and TLC.

Formation of Leptosin K (**4**) *and Derivative* (**11**) *from Leptosin* K_2 (**5**). — According to the procedure described for **6**, **5** (19 mg) was treated with triphenylphosphine (8 mg) at 60°C for 5 hr to yield **4** (1.1 mg) and **11** (2.3 mg), the former being identified by ^1H NMR, CD and TLC. **11** was obtained as a pale yellow powder, m.p. 233–235°C, $[\alpha]_D$ +179 (*c* 0.17 in $CHCl_3$); λ_{max} (EtOH)/nm 222 (log ε 4.77), 240 (4.27), 276 (4.04), 285 (4.09) and 292 (4.08); υ_{max} (KBr)/cm^{-1} 3503 (OH, NH), 1681, 1667 (CON),1607 and 1595 (Ar. C-C); δ_H ($CDCl_3$) 1.42 (3H, d, *J* 6.8 Hz, 16-H_3), 1.45 (3H, d, *J* 6.8 Hz, 15-H_3), 2.70 (1H, heptet, *J* 6.8 Hz, 14-H), 3.08 (3H, s, 13-H_3), 3.56 (1H, br s, 11-OH), 5.34 (1H, s, 11-H), 5.94 (1H, s, 5a-H), 6.05 (1H, s, 6-H), 6.77 (1H, dd, *J* 7.8 and 1.0 Hz, 7-H), 6.85 (1H, td, *J* 7.8, 1.0 Hz, 9-H), 7.08 (1H, td, *J* 7.5 and 1.0 Hz, 5'-H), 7.09 (1H, d, *J* 2.7 Hz, 2'-H), 7.20 (1H, td, *J* 7.8 and 1.0 Hz, 8-H), 7.21 (1H, td, *J* 7.5 and 1.0 Hz, 6'-H), 7.36 (1H, dd, *J* 7.5 and 1.0 Hz, 7'-H), 7.55 (1H, dd, *J* 7.5 and 1.0 Hz, 4'-H), 7.61 (1H, dd, *J* 7.8 and 1.0 Hz, 10-H) and 8.12 (1H, br d, *J* 2.7 Hz, 1'-H); *m/z* (FAB) 493 (MH^+); CD λ (*c* 3.00 x10^{-5} M in EtOH)/nm 231 (Δε -23.7), 268 (+8.4) and 298 (-5.1).

Bis (methylsulfanyl) Derivative **12**. This was derived from leptosins A–C (**1**–**3**), and its spectral data was reported except for CD data, previously;[5] CD λ (*c* 2.99x10^{-5} M in EtOH)/nm 224 (Δε +26.9), 244 (+16.7), 269 (-1.0), 281 (-0.5), 307 (-3.6), 337 (0) and 365 (-0.5).

X-Ray Crystallography of Leptosin K (**4**). — A colorless prismatic crystal of $C_{34}H_{36}N_6O_6S_4 \cdot 1/2C_4H_8O_2$ having approximate dimensions of 0.030 x 0.200 x 0.300 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochramated Cu-Kα radiation. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $40.54 < 2\theta < 46.64°$: Space group *P* 1, *a* = 14.019(2), *b* = 20.084(2), *c* = 13.691(1) Å, α = 100.31(1), β = 90.11(1), γ = 104.60(1)° , *V* = 3665.5(6) $Å^3$, *Z* = 4, d_x = 1.44 g/cm^3, μ = 28.7 cm^{-1} for Cu-Kα radiation. The data were collected at a temperature of 23 ±1°C using the ω - 2θ scan technique to a maximum 2θ value of 110.2° . Scans of

(1.52+0.30 tanθ)° were made at a speed of 8.0° / min (in omega). Of the 9306 reflections which were collected, 8876 were unique (R*int* = 0.045). The intensities of three representative reflection were measured after every 150 reflections. Over the couse of data collection, the standards decreased by -5.9%; a linear correction factor was applied. The structure was solved by direct methods[11] and expanded using Fourier techniques[12]. Most of the hydrogen atoms were generated geometrically and included in the refinement. Non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 10102 observed reflections ($I > 1.00\sigma(I)$) and 2058 variable parameters and converged (largest parameter was 3.50 times its esd) to $R = 0.067$, $R_w = 0.071$, where $w = 1/\sigma^2(Fo)$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.79 and -0.40 e Å^{-3}, respectively. Atom scattering factors were taken from Cromer and Waber.[13] All calculations were performed using the teXsan[14] crystallographic software package of Molecular Structure Corporation.

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