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Discodermins F-H, Cytotoxic and Antimicrobial Tetradecapeptides from the Marine Sponge *Discodermia kiiensis*: Structure Revision of Discodermins A-D[1]

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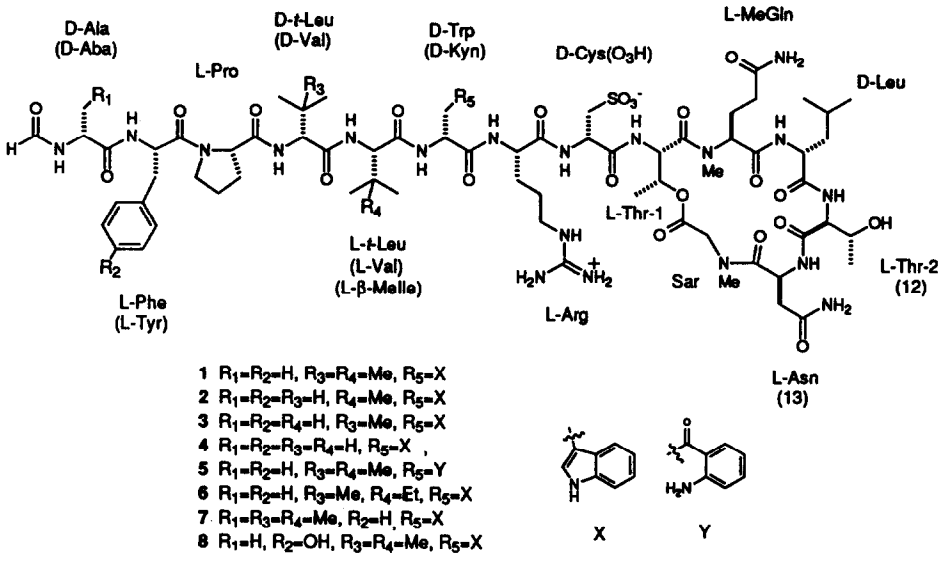
Abstract: Three new cytotoxic and antimicrobial peptides, discodermins F (**6**), G (**7**), and H (**8**), have been isolated from the marine sponge *Discodermia kiiensis*. The structures of **6-8** were determined by interpretation of spectral data and chemical degradation. Structures of discodermins A-D were revised on the basis of NMR data and protein sequence analysis.

Sponges of the genus *Discodermia* are a rich source of peptidic metabolites, e.g., calyculins from *D. calyx*,[2] discodermins A-E (**1-5**) and discokiolides from *D. kiiensis*,[3,4] and polydiscamide A from *Discodermia* sp.[5] Recently, we reported isolation and structure elucidation of discodermin E from *D. kiiensis* collected off Atami.[6] Further investigation of the extract of this sponge resulted in the isolation of three new congeners, discodermins F (**6**), G (**7**), and H (**8**). The sequence of the 12th and 13th residues in discodermins E-H was reversed from that in discodermin A. This discrepancy prompted us to reexamine the structures of discodermins A-D, which led to revision of their structures. In this paper, we describe isolation and structure elucidation of the new compounds, discodermins F-H, along with structure revision of discodermins A-D.

The water-soluble portion of the EtOH extract of the frozen sponge (5.0 kg) was partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was fractionated by ODS flash chromatography with aqueous MeOH. The active fraction eluted with 80% MeOH was separated on Sephadex LH-20, followed by ODS MPLC with 67% MeOH to yield seven fractions which were further purified by reversed phase HPLC with MeCN/H_2O (33.5:66.5) containing 0.05% TFA to yield **6-8** (**6**, 12 mg, 2.4 x 10^{-4}% yield wet weight; **7**, 10 mg, 2.0 x 10^{-4} %; **8**, 8 mg, 1.6 x 10^{-4} %) together with the known discodermins A-E (**1-5**).

Discodermin F (**6**) had a molecular formula of $C_{78}H_{119}N_{20}O_{22}S$, which was determined by HRFABMS. IR bands at 3340, 1735, and 1640 cm^{-1} were attributed to hydroxyl, ester, and amide groups, respectively. A negative ninhydrin reaction indicated its *N*-terminus to be blocked. Standard amino acid analysis of the acid hydrolysate revealed the presence of 10 amino acids, *viz.* Ala, Phe, Pro, Trp, Arg, Cys(O_3H), Leu, Asn, and Thr (2 residues). In addition, four unusual amino acids were observed in the amino acid analysis, which were identified as *t*-Leu, β-MeIle (β-methylisoleucine), MeGln (*N*-methylglutamine), and Sar (*N*-methylglycine) on the basis of HOHAHA[7] and NOESY[8] data. The UV spectrum [λ_{max} 275 nm (ε 3,880), 282 (4,070), 290 (3,390)] was consistent with Trp.

^{1}H NMR data of β-MeIle residue [δ 4.17 (d, *J*=8.1 Hz), 1.04 (2H, q, *J*=7.3 Hz), 0.72 (3H, s), 0.62 (3H, t, *J*=7.3 Hz), and 0.60 (3H, s)] were comparable to those reported.[5] The presence of a *t*-Leu residue was evident from a 9H-singlet methyl signal (δ 0.90) and an α methine proton [δ 4.51 (d, 9.5 Hz)] coupled to an



amide proton. *N*-Methyl groups in MeGln and Sar residues were assigned on the basis of intra-residual NOESY cross peaks. Substitution patterns of the side chain in MeGln and Asn residues were inferred from the HOHAHA and NOESY spectra: two pairs of mutually coupled primary amide protons (δ 7.18/6.65, 7.28/6.71) exhibited pertinent NOESY cross peaks with β- or γ-protons of Asn and MeGln residues. A formamide group [δ_H 7.89, δ_C 160.3] was observed in the NMR spectra.

Amino acid sequence of discodermin F was deduced by interpretation of the NOESY spectrum. Inter-residual cross peaks observed in NOESY experiments are as follows: CHO/Ala NH; Ala Hα/Phe NH; Phe NH, Hα/Pro $H_2\delta$; Pro $H_2\beta$/*t*-Leu NH; *t*-Leu NH, Hα/β-MeIle NH; β-MeIle Hα/Trp NH; Trp NH, Hα/Arg NH; Arg NH, Hα/Cys(O_3H) NH; Cys(O_3H) NH, Hα/Thr-1 NH; Thr-1 Hα/MeGln NMe; MeGln Hα/Leu NH; Leu Hα/Thr-2 NH; Thr-2 NH, Hα/Asn NH; Asn NH, Hα/Sar NMe. These data led to the sequence of discodermin F (**6**).

The remaining connection to be clarified was in the macrocyclic lactone between the carbonyl group of the C-terminal Sar residue and one of the hydroxyl groups of the two Thr residues. The β-proton of Thr-1 resonated at 5.17 ppm, thereby revealing participation of the hydroxyl group of this residue in the lactone formation. This was confirmed by an HMBC[9] cross peak between Thr-1 βH and Sar carbonyl carbon.

Absolute stereochemistry of the amino acid residues was determined by HPLC analysis of the acid hydrolysate derivatized with Marfey's reagent.[10] All amino acids except β-MeIle had absolute configurations identical with those in discodermin A. β-MeIle was determined to be L by Marfey analysis. β-MeIle in the acid hydrolysate co-eluted with L-β-MeIle isolated from polytheonamide B.[11]

Discodermin G (**7**) gave an $(M+H)^+$ ion at *m/z* 1719.8600 in the HRFAB mass spectrum, establishing a molecular formula of $C_{78}H_{119}N_{20}O_{22}S$. The ^1H NMR spectrum readily indicated that discodermin G contained an α-aminobutyric acid (Aba) residue in place of the Ala residue in discodermin A. Again, the amino acid sequence was determined by interpretation of NOESY data. Chirality of the amino acid residues was determined by Marfey analysis of the acid hydrolysate. The stereochemistry of the two *t*-Leu residues was determined by

Table 1. ^{1}H and ^{13}C NMR Data of Discodermin F (6) in DMSO-d_6[a]

Position	^{1}H (mult., J Hz)	^{13}C[b]	HMBC	Position	^{1}H (mult., J Hz)	^{13}C[b]	HMBC
CHO	7.89 (s)	160.3	Ala α, Ala NH	NH	7.90 (d, 7.3)		
Ala				CO		170.6	β, Cys(O_3H) NH
α	4.32 (m)	46.2	CHO, β				
β	0.90 (3H, d, 6.2)	18.5	α	δ-NH	7.37 (m)		
NH	8.10 (br d, 6.7)			Cys(O_3H)			
CO		171.4	α, β, Phe NH	α	4.61 (m)	50.7	
Phe				β	2.94 (m)	52.2	α
α	4.71 (m)	51.8	β		2.98 (m)		
β	2.72 (br d, 13.9)	36.9	2', 6'	NH	8.32 (d, 7.1)		
	3.01 (m)			CO		170.4	α
1'		137.6	β, 3'/5'	Thr-1			
2'/6'	7.26 (2H, d, 7.2)	129.4	β, 4', 2'/6'	α	4.88 (d, 8.3) [4.86]	51.3	γ
3'/5'	7.23 (2H, t, 7.2)	128.2	3'/5'	β	5.17 (q, 6.7) [5.04]	69.0	γ
4'	7.16 (t, 7.2)	126.3	2'/6'	γ	1.18 (3H, d, 6.6)	17.1	α
NH	8.38 (d, 8.5)			NH	8.00 (d, 8.3)		
CO		170.7	α	CO		169.2	α, MeGln Me
Pro				MeGln			
α	4.53 (m)	59.3		α	5.02 (m)	54.2	
β	1.86 (m)	29.4		β	1.79 (m)	24.8	
	2.10 (m)				1.91 (m)		
γ	1.86 (2H, m)	24.0		γ	1.93 (2H, m)	31.5	β, γ-NH_2
δ	3.62 (2H, m)	46.7		NMe	3.06 (s)	30.8	
CO		170.7	α, *t*-Leu NH	CO		169.9	α, Leu NH
t-Leu				γ-NH_2	7.18 (brs)		
α	4.51 (d, 9.5)	59.2	γ		6.65 (brs)		
β		35.3	α, γ	CO		173.4	γ
γ	0.90 (9H, s)	26.7	α, γ	Leu			
NH	7.67 (d, 9.5)			α	4.47 (m)	51.0	β, γ,
CO		170.1	α, β-MeIle NH	β	1.43 (m) [1.44]	41.8	α, γ, δ
β-MeIle					1.54 (m) [1.55]		
α	4.17 (d, 8.1)	59.5	γ'	γ	1.54 (m) [1.55]	24.1	β, δ
β		36.3	α, γ, γ', δ	δ	0.89 (6H, m)	22.2	β, γ, δ
γ	1.04 (2H, q, 7.3)	31.2	γ', γ', δ		[0.83]		
δ	0.62 (3H, t, 7.3)	7.8		NH	7.62 (d, 6.8)		
γ'	0.60 (3H, s)	22.8	γ''	CO		172.1	α, Thr-2 NH
γ'	0.72 (3H, s)	23.1	γ', α	Thr-2			
NH	7.79 (d, 8.1)			α	3.95 (dd, 8.2, 4.1)	60.4	γ
CO		170.0	α, Trp NH		[3.85]		
Trp				β	4.02 (m)	65.3	γ
α	4.58 (m)	53.8	β	γ	1.04 (3H, d, 6.3)	20.2	α
β	2.93 (m)	27.6	α		[0.96]		
	3.14 (m)			NH	7.90 (d, 8.2)		
1'	10.6 (s)			CO		169.9	α
2'	7.14 (brs)	124.1	β	Asn			
3'		109.9	β, 2' 4'	α	5.00 (m)	45.6	
3'a		127.1	β, 2', 5', 7'	β	2.08 (m) [2.22]	36.8	β-NH_2
4'	7.56 (d, 7.3)	118.4	6'		2.67 (dd, 15.2, 9.3)		
5'	6.95 (t, 7.3)	118.2	7'		[2.72]		
6'	7.03 (t, 7.3)	120.8	4'	NH	7.54 (d, 9.5)		
7'	7.32 (d, 7.3)	111.3	5'	CO		169.5	α, Sar Me
7'a		137.5	2', 4', 6'	β-NH_2	7.28 (brs)		
NH	8.21 (brs)				6.71 (brs)		
CO		171.2	α, Arg NH	CO		171.8	β
Arg[c]				Sar			
α	4.37 (m)	51.7	β	α	3.50 (d, 17.3)	49.2	NMe
β	1.60 (2H, brs)	28.6	α		4.44 (d, 17.3)		
γ	1.28 (m)	23.4		NMe	2.75 (s)	35.2	α
	1.33 (m)			CO		167.4	α, Thr–1 β
δ	2.96 (2H, m)	40.2	β, γ				

a: Discodermin H adopts two conformers in DMSO-d_6, giving two sets of signals in a ratio of 4:1. The signals for the minor conformers were bracketed.

b: ^{13}C chemical shifts were determined by tracing the HMQC and HMBC spectra.

c: Guanidyl carbon did not give a cross peak in the HMBC spectrum.

Table 2. ^{1}H and ^{13}C NMR Data of Discodermin A (1) in DMSO-d_6[a]

Position	^{1}H (mult., J Hz)	^{13}C[b]	HMBC	Position	^{1}H (mult., J Hz)	^{13}C[b]	HMBC
CHO	7.89 (s)	160.4	Ala α, Ala NH	Cys(O_3H)			
Ala				α	4.62 (m)	50.5	β
α	4.32 (m)	46.3	β	β	2.94 (m)	52.4	α
β	0.89 (3H, d, 7.0)	18.3	α		2.98 (m)		
NH	8.11 (d, 8.1)			NH	8.31 (d, 7.7)		
CO		171.3	α, β, Phe NH	CO		170.8	α, β, Thr-1 NH
Phe				Thr-1			
α	4.70 (m)	51.7	β	α	4.87 (d, 8.9)	51.5	γ
β	2.72 (br d, 13.6)	36.9		β	5.18 (q, 6.6)[5.05]	68.9	γ
	2.99 (m)			γ	1.17 (3H, d, 6.9)	17.1	
1'		137.5	α, β, 3'/5'		[1.16]		
2'/6'	7.25 (2H, d, 7.1)	129.3	β, 4', 2'/6'	NH	8.03 (d, 6.9)		
3'/5'	7.22 (2H, t, 7.1)	128.0	3'/5'	CO		169.2	α, β, MeGln Me
4'	7.16 (t, 7.0)	126.2	2'/6'				
NH	8.38 (d, 8.6)						
CO		169.8	α, β	MeGln			
Pro				α	4.97 (m)	54.4	
α	4.53 (m)	59.3		β	1.80 (m)	24.6	
β	1.92 (m)	29.5			1.92 (m)		
	2.10 (m)			γ	1.98 (2H, m)	31.5	β, γ-NH_2
γ	1.85 (2H, m)	23.8		NMe	3.05 (s)	30.7	
δ	3.63 (2H, m)	46.9		CO		170.1	α, β, Leu NH, Leu α
CO		170.7	α, *t*-Leu NH				
t-Leu-1				γ-NH_2	7.17 (brs)		
α	4.51 (d, 9.9)	59.3	γ		6.66 (brs)		
β		35.4	α, γ	CO		173.5	γ
γ	0.89 (9H, s)	26.6	α, γ	Leu			
NH	7.69 (d, 9.9)			α	4.45 (m)	50.9	β, γ
CO		169.9	α, *t*-Leu NH	β	1.42 (m) [1.43]	41.8	α, γ, δ
t-Leu-2					1.54 (m) [1.58]		
α	4.12 (d, 7.4)	60.9	γ	γ	1.53 (m) [1.58]	24.0	β, δ
β		33.6	α, γ	δ	0.88 (6H, m)	22.5	β, γ, δ
γ	0.72 (9H, s)	26.6	α, γ		[0.82]		
NH	7.83 (d, 7.4)			NH	7.66 (d, 7.8)		
CO		170.2	α, Trp NH	CO		172.1	α, Thr-2 NH, Thr-2 α
Trp							
α	4.58 (m)	53.9	β	Thr-2			
β	2.94 (m)	27.6	α	α	3.95 (dd, 8.2, 4.1)	60.3	γ
	3.14 (m)				[3.86]		
1'	10.64 (s)			β	4.02 (m)	65.4	γ
2'	7.13 (brs)	123.9	β	γ	1.04 (3H, d, 6.3)	20.1	
3'		109.7	β, 2', 4'		[1.01]		
3'a		127.0	β, 2', 5', 7'	NH	7.90 (d, 8.2)		
4'	7.57 (d, 7.3)	118.2	6'	CO		170.0	α
5'	6.94 (t, 7.3)	118.0	7'	Asn			
6'	7.02 (t, 7.3)	120.9	4'	α	5.00 (m)	46.0	
7'	7.31 (d, 7.3)	111.3	5'	β	2.08 (m) [2.24]	36.9	β-NH_2
7'a		137.4	2', 4', 6'		2.67 (dd, 15.2, 9.3)		
NH	8.23 (brs)				[2.72]		
CO		171.1	α, Arg NH	NH	7.54 (d, 9.5)		
Arg[c]				CO		169.3	α, Sar Me
α	4.34 (m)	51.8	β	β-NH_2	7.31 (brs)		
β	1.58 (2H, brs)	28.7	α		6.73 (brs)		
γ	1.28 (m)	23.5		CO		172.1	β
	1.32 (m)			Sar			
δ	2.94 (2H, m)	40.3	γ	α	3.49 (d, 17.3)	49.1	NMe
NH	7.93 (br d, 8.3)				4.44 (d, 17.3)		
CO		170.7	α, Cys(O_3H) NH	NMe	2.75 (s)	35.1	α
				CO		167.6	α, Thr-1 β
δ-NH	7.36 (m)						

a: Discodermin A adopts two conformers in DMSO-d_6, giving two sets of signals in a ratio of 4:1. The signals for the minor conformers were bracketed.

b: ^{13}C chemical shifts were determined by tracing the HMQC and HMBC spectra.

c: Guanidyl carbon did not give a cross peak in the HMBC spectrum.

combining Edman degradation with Marfey analysis; deformylated discodermin G was subjected to four cycles of the Edman degradation followed by Marfey analysis of the acid hydrolysate. The peak heights of L- and D-*t*-Leu were 5:1, thereby indicating that the 4th and 5th residues from the *N*-terminus were D-*t*-Leu and L-*t*-Leu, respectively.

The molecular formula of discodermin H (8) was determined to be $C_{77}H_{117}N_{20}O_{23}S$ on the basis of the HRFAB mass spectrum. ^1H and ^{13}C NMR data indicated that discodermin H had a Tyr residue in place of the Phe residue in discodermin A. The sequencing of amino acid residues was accomplished by interpretation of NOESY data. The absolute configuration of amino acid residues was determined by Marfey analysis of the acid hydrolysate. The stereochemistry of the two *t*-Leu residues was determined as for discodermin G.

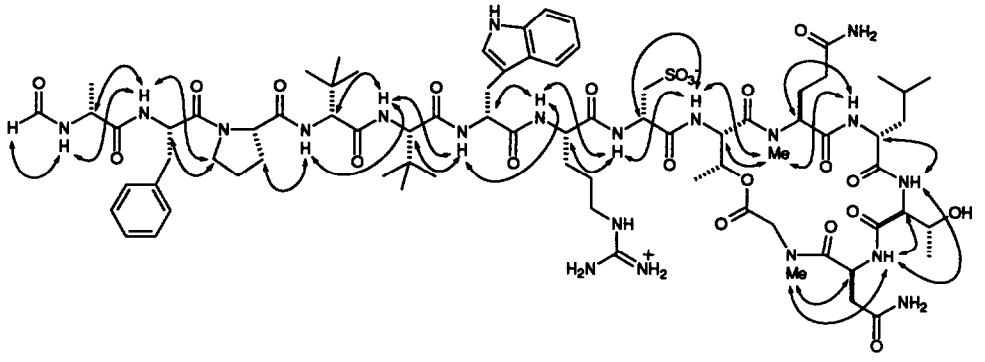


Fig. 1. Correlations obtained from NOESY experiment for discodermin A (1)

Since the sequence of the 12th and 13th residues in discodermins A-D (Asn and Thr, respectively) was reversed from that in discodermins E-H, we reexamined the structure of discodermin A.[12] After assignment of all ^1H and ^{13}C NMR signals to each amino acid (Table 2), NOESY and HMBC data were carefully analyzed, which indicated that the order of the 12th and 13th residues had to be reversed. To substantiate this finding, discodermin A was subjected to deformylation, methanolysis, and sequence analysis by a gas-phase protein sequencer, which unambiguously showed that the 12th and 13th residues were Thr and Asn, respectively. Structures of discodermins B-D must similarly be revised, because they gave identical octapeptide fragments as discodermin A, which contained residues 7-14, upon treatment with BNPS-skatol.[3]

Discodermins F, G, and H inhibited the growth of bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonus aeruginosa*) at 5-10 μg/disk, and fungi (*Mortierella ramanniana, Candida albicans,* and *Penicillium chrysogenum*) at 25 μg/disk. They were cytotoxic against P388 leukemia cells at IC_{50}'s of 0.1, 0.4, and 0.1 μg/mL, and inhibited the development of starfish (*Asterina pectinifera*) embryos at 10, 20, and 10 μg/mL, respectively.

Recently, similar cyclic peptides have been isolated from the marine sponge *Halichondria cylindrata*,[1] therefore discodermins are likely produced by microbial symbionts.

EXPERIMENTAL

General methods. Uv spectra were recorded on a Hitachi 330 spectrophotometer. Infrared spectra

were measured with a JASCO IR-G infrared spectrometer. ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker AM-600 or JEOL GMX-500 NMR spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to solvent peaks: δ_H 2.49 and δ_C 39.5 for DMSO-d_6. Mass spectra were measured on a JEOL JMX-SX102 mass spectrometer. High resolution FAB mass spectra were measured using a dual target inlet probe. Optical rotations were determined with a JASCO DIP-371 digital polarimeter. Amino acid analysis was performed with a Hitachi L-8500A amino acid autoanalyzer. Automatic protein sequence analysis was carried out with an Applied Biosystems 476A protein sequencer and a 120A PTH analyzer.

Collection and Isolation. The sponge samples were collected by Scuba at depths of 10-20 m off Atami, 90 km southwest of Tokyo, and kept frozen until used. The frozen sponge (5.0 kg, wet weight) was extracted by blending with EtOH (1.5 L x 3) and 70% EtOH (1.5 L). The combined extracts were concentrated and partitioned between H_2O (800 mL) and Et_2O (800 mL x 3). The water-soluble portion was further extracted with *n*-BuOH (800 mL x 3). The *n*-BuOH fraction (11.8 g) was subjected to flash chromatography on an ODS column (70/230 mesh) with H_2O, 40%, 80%, and 100% MeOH. The 80% MeOH fraction (4.32 g) was fractionated by gel filtration on Sephadex LH-20 with MeOH to afford the major cytotoxic fraction (3.3 g). This fraction was further separated by ODS-MPLC (3.0 x 100 cm) with 67% MeOH to yield seven active fractions (B2-1, 40 mg; B2-2, 124 mg; B2-3, 15 mg; B2-4, 11 mg; B2-5, 2.34 g; B2-6, 17 mg; B2-7, 20 mg). These fractions were finally purified by reverse phase HPLC [ODS, 10 x 250 mm; MeCN/H_2O (33.5:66.5) containing 0.05% TFA; flow rate 2.0 mL/min; UV detection at 210 nm] to yield discodermin D (**4**, 32 mg, 6.4 x 10^{-4}% yield) from fraction B2-1; discodermin B (**2**, 55 mg, 1.1 x 10^{-3}%) and discodermin C (**3**, 48 mg, 9.8 x 10^{-4}%) from fraction B2-2; discodermin H (**8**, 8 mg, 1.6 x 10^{-4}%) from B2-3; discodermin E (**5**, 3.5 mg, 7.0 x 10^{-5}%) from B2-4; discodermin A (**1**, 2.1 g, 0.42%) from B2-5; discodermin G (**7**, 10 mg, 2.0 x 10^{-4}%) from B2-6; discodermin F (**6** , 12 mg, 2.4 x 10^{-4}%) from B2-7.

Discodermin F (6): colorless amorphous solid; $[\alpha]^{23}_D$ -6.7° (*c* 0.82, MeOH); IR (film) 3340, 3050, 2950, 1735, 1640, 1540, 1420, 1180 cm^{-1}; UV λ_{max} (MeOH) 217 nm (ε 42,100), 275 (3,880), 282 (4,070), 290 (3,390); FABMS *m/z*1741 $(M+Na)^+$, 1719 $(M+H)^+$, 1637, 1473, 1078, 969, 898, 595, 391, 344; HRFABMS *m/z* 1719.8635 calcd. for $C_{78}H_{119}N_{20}O_{22}S$ (Δ +10.7 mmu); ^{1}H NMR and ^{13}C NMR (DMSO-d_6) see Table 1.

Discodermin G (7): colorless amorphous solid; $[\alpha]^{23}_D$ -6.8° (*c* 0.64, MeOH); IR (fim) 3340 (br), 3050, 2950, 1735, 1640, 1540, 1420, 1180 cm^{-1}; UV λ_{max} (MeOH) 217 nm (ε 49,200), 275 (3,970), 282 (4,230), 290 (3,640); FABMS *m/z*1719 $(M+H)^+$, 1624, 1459, 1049, 969, 898, 707, 407, 358, 344; HRFABMS *m/z* 1719.8600 calcd. for $C_{78}H_{119}N_{20}O_{22}S$ (Δ +7.1 mmu); ^{1}H NMR (DMSO-d_6) (signals for the minor conformer were shown in brackets) **δ CHO**: 7.94 (s); **Aba**: 4.28 (m, αH), 1.28 (2H, m, βH), 0.48 (3H, t, *J*=7.4 Hz, γH), 8.06 (d, 8.7, NH); **Phe**: 4.71 (m, αH), 2.72 (br d, 14.0, βH), 2.99 (m, βH), 7.27 (2H, d, 7.4, H2' and H6'), 7.23 (2H, t, 7.4, H3' and H5'), 7.16 (t, 7,4, H4'), 8.47 (d, 8.5, NH); **Pro**: 4.54 (m, αH), 1.87 (m, βH), 2.09 (m, βH), 1.86 (2H, m, γH), 3.68 (2H, m, δH); ***t*-Leu-1**: 4.51 (d, 9.2, αH), 0.89 (9H, s, γH), 7.68 (d, 9.2, NH); ***t*-Leu-2**: 4.14 (d, 7.9, αH), 0.72 (9H, s, γH), 7.83 (d, 7.9, NH); **Trp**: 4.58 (m, αH), 2.92 (m, βH) and 3.13 (dd, 13.9, 5.1, βH), 10.6 (s, H1'), 7.14 (d, 1.9, H2'), 7.58 (d, 7.5, H4'), 6.95 (t, 7.5, H5'), 7.03 (t, 7.5, H6'), 7.31 (d, 7.5, H7'), 8.23 (d, 7.9, NH); **Arg**: 4.36 (m, αH), 1.60 (2H, m, βH), 1.28 (m, γH), 1.33 (m, γH), 2.95 (m, δH), 2.98 (m, δH), 7.95 (d, 7.9, NH), 7.38 (m, δ-NH); **Cys(O_3H)**: 4.61 (m, αH), 2.94 (m, βH), 2.98 (m, βH), 8.32 (d, 6.8, NH); **Thr-1**: 4.87 (d, 8.0, αH) [4.73], 5.16 (q, 6.5, βH) [5.04], 1.17 (3H, d, 6.5, γH), 8.01 (d, 8.0, NH); **MeGln**: 5.01 (m, αH), 1.80 (m, βH), 1.92 (m, βH), 1.95

(2H, br m, γH), 3.06 (3H, s, NMe), 7.19 (brs, $CONH_2$), 6.67 (brs, $CONH_2$); **Leu**: 4.46 (m, αH), 1.43 (m, βH) [1.46], 1.53 (m, βH) 1.59], 1.53 (m, γH) [1.59], 0.89 (6H, m, δH) [0.82], 7.64 (d, 6.8, NH); **Thr-2**: 3.95 (dd, 8.1, 4.1, αH) [3.85], 4.02 (m, βH), 1.04 (3H, d, 6.3, γH) [0.95], 7.91 (d, 8.1, NH); **Asn**: 4.99 (m, αH), 2.08 (m, βH) [2.22], 2.67 (dd, 15.2, 9.3, βH) [2.72], 7.56 (d, 9.8, NH), 7.29 (brs, $CONH_2$), 6.73 (brs, $CONH_2$); **Sar**: 3.53 (d, 17.2, αH) and 4.44 (d, 17.2, αH), 2.75 (3H, s, NMe).

Discodermin H (8): colorless amorphous solid; $[\alpha]^{23}_D$ -5.8° (*c* 0.62, MeOH); IR (film) 3340 (br), 3050, 2950, 1740, 1645, 1540, 1420, 1185 cm^{-1}; UV λ_{max} (MeOH) 216 nm (sh), 226 (sh), 274 (ε 4,510), 280 (4,730), 289 (3,680); FABMS *m/z* 1721(M+H)$^+$, 1641, 1459, 1136, 971, 945, 853, 360; HRFABMS *m/z* 1721.8236 calcd. for $C_{77}H_{117}N_{20}O_{23}S$ (Δ -8.5 mmu); ^1H NMR (DMSO-d_6) δ **CHO**: 7.93 (s); **Ala**: 4.33 (m, αH), 0.95 (3H, d, 6.9, βH), 7.62 (d, 7.9, NH); **Tyr**: 4.60 (m, αH), 2.61 (dd, 13.7, 10.6, βH), 2.87 (m, βH), 7.04 (2H, d, 8.4, H2' and H6'), 6.61 (2H, d, 8.4, H3' and H5'), 8.47 (d, 8.3, NH); **Pro**: 4.52 (m, αH), 1.85 (m, βH), 2.09 (m, βH), 1.85 (2H, m, γH), 3.65 (2H, m, δH); ***t*-Leu-1**: 4.51 (d, 9.3, αH), 0.89 (9H, s, γH), 7.66 (d, 9.3, NH); ***t*-Leu-2**: 4.13 (d, 7.9, αH), 0.71 (9H, s, γH), 7.84 (d, 7.9, NH); **Trp**: 4.57 (m, αH), 2.93 (m, βH), 3.14 (dd, 14.2, 5.4, βH), 10.6 (s, H1'), 7.14 (d, 1.9, H2'), 7.58 (d, 7.3, H4'), 6.95 (t, 7.3, H5'), 7.03 (t, 7.3, H6'), 7.31 (d, 7.3, H7'), 8.23 (d, 7.5, NH); **Arg**: 4.35 (m, αH), 1.59 (2H, m, βH), 1.29 (m, γH), 1.33 (m, γH), 2.95 (m, δH), 2.98 (m, δH), 7.94 (d, 8.0, NH), 7.38 (m, δ-NH); **Cys(O_3H)**: 4.60 (m, αH), 2.94 (m, βH), 2.98 (m, βH), 8.33 (d, 6.7 NH); **Thr-1**: 4.87 (d, 8.2, αH), 5.16 (q, 6.3, βH) [5.04], 1.17 (3H, d, 6.5, γH), 8.01 (d, 8.2, NH); **MeGln**: 5.02 (m, αH), 1.80 (m, βH), 1.92 (m, βH), 1.95 (2H, br m, γH), 3.06 (3H, s, NMe), 7.19 (brs, $CONH_2$), 6.66 (brs, $CONH_2$); **Leu**: 4.46 (m, αH), 1.43 (m, βH) [1.46], 1.54 (m, βH) [1.60], 1.54 (m, γH) [1.60], 0.89 (6H, m, δH) [0.82], 7.65(d, 6.9, NH); **Thr-2**: 3.95 (dd, 8.1, 4.0, αH) [3.85], 4.02 (m, βH), 1.03 (3H, d, 6.3, γH) [0.96], 7.91 (d, 7.9, NH); **Asn**: 5.00 (m, αH), 2.09 (m, βH) [2.22], 2.67 (dd, 15.3, 9.5, βH) [2.72], 7.56 (d, 9.0, NH), 7.29 (brs, $CONH_2$), 6.73 (brs, $CONH_2$); **Sar**: 3.48 (d, 16.7, αH), 4.44 (d, 16.7, αH), 2.75 (3H, s, NMe).

Sequence analysis of discodermin A by automatic protein sequencer. Discodermin A (100 μg) was deformylated with 10% HCl/MeOH (0.3 mL) at room temperature for 5h. After removal of HCl, the reaction mixture was treated with 0.1 N NaOH and subjected to a protein sequencer.

Marfey analysis of the acid hydrolysate. Discodermin F (100 μg) was dissolved in 6N HCl (100 μL) and heated at 110 °C for 18h. After removal of HCl in vacuo, the residue was treated with 10% acetone solution of FADD (50 μL) in 1M $NaHCO_3$ (100 μL) at 80-90 °C for 3 min followed by neutralization with 2N HCl (50 μL). The reaction mixture was diluted with 50% MeCN, and subjected to HPLC analysis: column; ODS, 5 μm, 4.6 x 250 mm, eluent; Solvent A: MeCN/5 mM $(Et_3NH)_3PO_4$, pH 3.0 (1:9), Solvent B: MeCN/5 mM $(Et_3NH)_3PO_4$, pH 3.0 (4:6): elution with solvent A for 10 min, followed by a gradient elution to A/B=50:50 in 35 min, a second gradient elution to solvent B in 35 min, and elution with solvent B for 20 min. The peaks were identified by co-injection with a DL-mixture of standard amino acids. Retention times (min) are given in parentheses. D-Cys(O_3H) (34.6), L-Arg (35.1), L-Thr (44.2), L-Asn (46.2), L-MeGln (55.6), L-Pro (59.2), D-Aba (63.1), D-Ala (65.4), L-Tyr (67.1), D-Trp and L-Phe (78.0), D-t-Leu (85.6), L-β-MeIle (86.4), D-Leu (87.8). Discodermin G and H were analyzed in the same way.

Determination of the configurations of two *t*-Leu residues in discodermins G and H. Deformylated discodermin G (100 μg) in 20 μL of PITC solution [ethanol/trimethylamine/H_2O/PITC (7:1:1:1, v/v)] was warmed for 8 min in a water bath at 50 °C. After cooling to room temperature, the reaction mixture was washed once with 400 μL of heptane/ethyl acetate (15:1), then twice with 400 μL of heptane/ethyl acetate

(7:1). The lower phase was freeze-dried, triturated with H_2O (40 μL) and benzene/acetonitrile (2:1, 400 μL). The suspesion was centrifuged; the lower phase was subjected to the same procedure three more times to give a decapeptide containing only one *t*-Leu residue. Marfey analysis of the acid hydrolysate of the decapeptide resulted in a 5:1 mixture of D-*t*-Leu and L-*t*-Leu. Discodermin H gave the same result when treated in the same way.

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12. The assignment of the 12th residue in discodermin A was previously established by applying the Edman-dansyl method to a BNPS-skatol fragment of the methyl ester of seco-acid, whereas the 13th residue was assigned by a process of elimination, because the C-terminus was assigned as Sar by hydrazinolysis.

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