Seven New Didemnins from the Marine Tunicate *Tridemnum solidum*

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Abstract: Seven new didemnins—didemnins M (1), N (2), X (3), and Y (4), nordidemnin N (5), epididemnin A_1 (6), and acyclodidemnin A (7)—were isolated from an extract of the Caribbean tunicate *Trididemnum solidum*. The structures of these compounds were assigned, based on FABMS, high-field NMR data, and chemical degradation studies. Biological activities of these peptides are also described.

Introduction

Cyclic depsipeptides didemnins A (**8**), B (**9**), and C (**10**) were isolated from the Caribbean tunicate *Trididemnum solidum* and reported in 1981 as antiviral−antitumor agents.[1] Later, didemnins D (**11**) and E (**12**), nordidemnins A (**13**) and B (**14**), and the formaldehyde adduct methylenedidemnin A (**15**),[1c] as well as didemnin G (**16**),[2] were also isolated from the same source. Because of their remarkable antitumor, antiviral, and immunosuppressive activities numerous chemical,[3] structural,[4] and biological[5] studies have been reported on the didemnins. Since earlier studies in our laboratory suggested that relatively simple modifications of the side chain or ring of **8** can increase activity, further systematic investigations of the structure−activity relationship of didemnins have been conducted, mainly by modifying **8** or **9** semisynthetically.[6] In addition, searches for new didemnins from the tunicate have continued since natural compounds can provide novel structural units which may not be obvious or readily accessible semisynthetically. In the present paper, isolation and structure elucidation of seven new compounds isolated from *T. solidum* are described: didemnins M (**1**), N (**2**), X (**3**), and Y (**4**), nordidemnin N (**5**), and epididemnin A_1 (**6**) as well as a ring-opened form of didemnin A, acyclodidemnin A (**7**).

Results

Didemnin B was the first marine natural product to enter clinical trials, and a large amount of extract of *T. solidum* was obtained during preparation of didemnin B (**9**) for phase I and phase II studies.[7] For phase I, 170 kg of the tunicate was extracted with toluene−MeOH (3:1) and the extract was partitioned between aqueous and organic phases.[2] The organic layer was chromatographed on a silica gel column to give crude **8**, **9**, and a polar fraction called "fraction A" (135 g), a MeOH−$CHCl_3$ (4:6) eluate. On-line LC/FAB mass analysis of fraction A (Figure 1) employing the moving belt technique[8] indicated that it contained a new didemnin (**3**) along with **8**, **9**, **11**, **12**, and **13**. Separation of fraction A afforded as major compounds **12**, **3**, **11**, and **4**, in order of abundance, as well as **8**, **9**, and **13**. Larger scale separation of fraction A was later conducted and afforded new didemnins M (**1**), N (**2**), X (**3**), and Y (**4**), nordidemnin N (**5**), and epididemnin A_1 (**6**) along with **11** and **12**.

Isolation of Didemnins X (3) and Y (4). A portion (9 g) of fraction A was further separated by using high-speed centrifugal countercurrent chromatography (HSCCC),[9] and each fraction containing didemnins was separately purified by using a polystyrene−divinylbenzene copolymer gel, reversed-phase (RP)HPLC, and normal-phase HPLC to give pure **3** (107.0 mg) and **4** (11.3 mg).

Isolation of Didemnins M (1) and N (2), Nordidemnin N (5), Epididemnin A_1 (6), and Acyclodidemnin A (7). Fraction A (128 g)[10] was partitioned between upper and lower layers of heptane−EtOAc−MeOH−H_2O (4:7:4:3). The lower layer afforded a peptide-rich fraction which was chromatographed on a silica gel column or by HSCCC. Subsequent chromato-

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(10) This "fraction A" was prepared at the Upjohn Co. during a large-scale separation of didemnin B for the phase II clinical trial.

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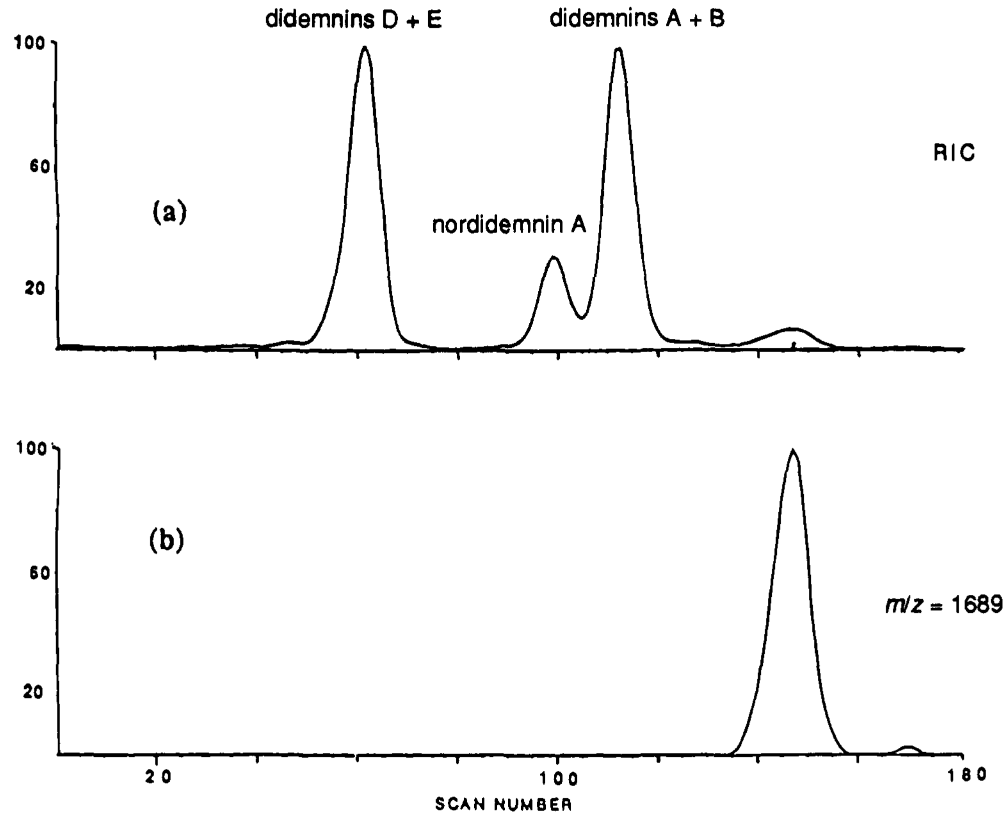

Figure 1. LC/FABMS of fraction A: (a) reconstructed ion chromatogram (RIC) and (b) single ion plot for *m/z* 1689 (M + Na) for didemnin X (**3**).

graphic purification using silica gel, Sephadex LH-20, and reversed-phase HPLC for the fractions which contained new peptides (monitored by FABMS and TLC) gave new didemnins M (**1**) and N (**2**, [Tyr[5]]didemnin A), nordidemnin N (**5**, [NorSta[1],Tyr[5]]didemnin A), epididemnin A$_1$ (**6**, [4-epiHip[2]]-didemnin A), and acyclodidemnin A (**7**, [acyclo[5,6]]didemnin A).

Structure of Didemnin X (3). In agreement with HR-FABMS data on the protonated molecular ion (1666.9533, Δ 2.6), the molecular formula of **3** was assigned as $C_{82}H_{131}N_{13}O_{23}$. Since **3** appeared as a complicated mixture of conformers, its NMR spectra were not well resolved, even at temperatures up to 90 °C in DMSO-d_6. However, the spectral pattern of **3** was very similar to those of **11** and **12**, suggesting that **3** shares the basic skeleton with other didemnins. Chiral GC analysis of the acid hydrolysate of **3** showed the same amino acid components found in **12**. Treatment of **3** with MeOH in the presence of sodium carbonate gave two major products, one of which had ^{1}H NMR, TLC, and optical rotation data identical with those of **9** and HRFABMS data in agreement with the formula $C_{57}H_{89}N_7O_{15}$ for **9**. The second product, **17** ($C_{26}H_{46}N_6O_9$, 587.3401, M + H, HRFABMS), containing the extended side chain of **3**, showed the presence of L-Glu on hydrolysis followed by chiral GC analysis. HRFABMS analysis of major fragment ions at *m/z* 555, 427, 299, and 188 for **17** showed sequential losses of two Gln ($C_5H_8N_2O_2$) units. HRFABMS on the ions at *m/z* 299 ($C_{15}H_{27}N_2O_4$) and 188 ($C_{10}H_{22}NO_2$) indicated the presence of a terminal C_{10} oxyacyl-Gln unit which allowed assignment of the sequence of **17** to be $C_{10}H_{19}O_2$-(L-Gln)$_3$-OCH_3 (Scheme 1).

Vigorous hydrolysis of **17** afforded a lipophilic compound, **18a**, with the molecular formula $C_{10}H_{20}O_3$ (HRFABMS). The structure of **18a** was assigned as 3-hydroxydecanoic acid by its ^{1}H NMR spectrum and by HREIMS data on the major fragmentation ion $C_3H_5O_3^+$ (*m/z* 89) and was confirmed by comparison of the spectral data for **18a** and its methyl ester **19a** with those of synthetic (*R*,*S*)-3-hydroxydecanoic acid (**18ab**) and its methyl ester **19ab** (the latter prepared by condensation of Meldrum's acid and octanoyl chloride).[11] Because the small amount of natural **19a** available did not allow an unambiguous assignment of the absolute stereochemistry at C-3 by optical rotation, **19a** was converted to its (+)-10-camphorsulfonate (**20a**)[12] in order to determine the stereochemistry by NMR. Optically pure methyl (3*R*)- and methyl (3*S*)-3-[(+)-(10-camphorsulfonyl)oxy]decanoate (**20a** and **20b**) were prepared by converting racemic **19ab** to epimeric (*R*)-methylbenzyl carbamates **21a** and **21b**,[13] which were easily separated by HPLC and then cleaved with trichlorosilane[14] to give optically pure methyl (*R*)-3-hydroxydecanoate (**19a**) ($[\alpha]^{20}_D$ −18.4°, lit.[15] −18.5°) and the corresponding *S* derivative **19b** ($[\alpha]^{20}_D$ +18.4°), respectively (Scheme 2). These esters were then converted to the camphorsulfonates **20a** and **20b**, respectively. The ^{1}H NMR spectra of **20a,b** showed very distinctive signals for the C-10′ position of the camphor unit, and the spectrum of the natural derivative **20a** is superimposable on that of the synthetic *R* derivative (Figure 2). Thus, the structure of **17** was assigned as (*R*)-(3-hydroxydecanoyl)-(Gln)$_3$-OCH_3. FABMS/CID/MS of **3** showed sequential losses corresponding to ($C_{10}H_{19}O_2$-Gln), Gln, Gln, Lac, and Pro units, indicating **17** is linked to the hydroxyl group of the lactyl unit of **9** (Scheme 3).

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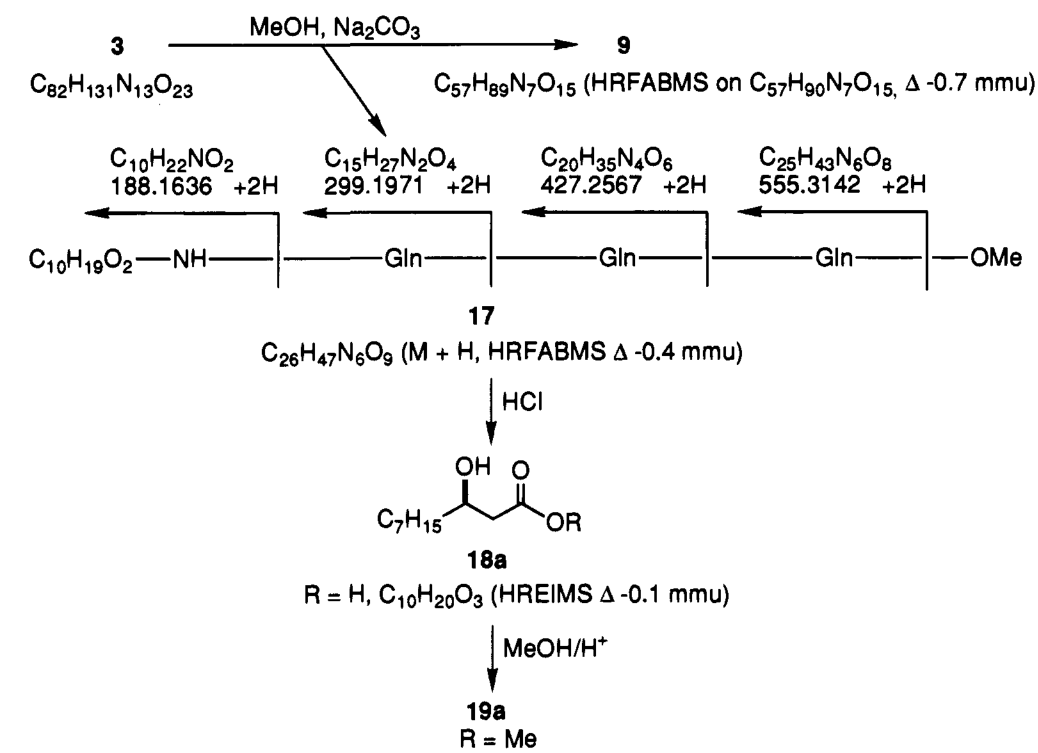
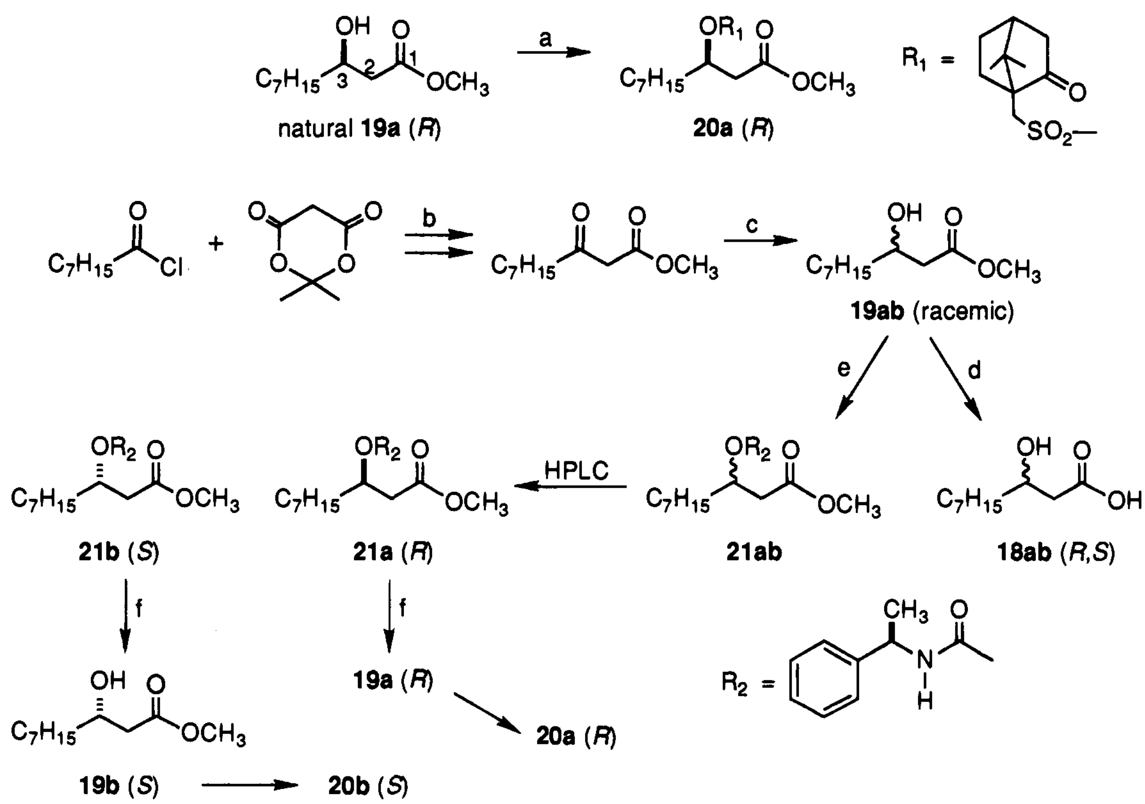
Scheme 1



Scheme 2[a]



[a] (a) 10-(+)-Camphorsulfonyl chloride, Pyr, room temperature. (b) (1) Pyr, CH_2Cl_2. (2) MeOH, reflux. (c) $NaBH_4$, THF−H_2O (56%). (d) 6 N NaOH, 110 °C, 1 min (83%). (e) (*R*)-α-Methylbenzyl isocyanate, CH_2Cl_2, Et_3N, reflux, 42 h (78%). (f) $SiHCl_3$, Et_3N, C_6H_6, 36 h, room temperature (33% **19b**, 54% **19a**).

Structure of Didemnin Y (4). The ^1H NMR spectra of **4** showed a pattern similar to that of **3**, with broad peaks, suggesting the two were related. Chiral GC of the derivatized acid hydrolysate ((TFA)OMe derivatives) of **4** gave results identical with those for **3**. The molecular formula, $C_{87}H_{139}N_{15}O_{25}$, was determined on the basis of HRFABMS data on the molecular ion at *m/z* 1795.0119 (M + H). The formulas of **3** and **4** differ by $C_5H_8N_2O_2$, corresponding to one glutaminyl unit. A FABMS/CID/MS spectrum of **4** on the molecular ion showed sequential losses for ($C_{10}H_{19}O_2$-Gln), Gln, Gln, Gln, Lac, and Pro units, indicating that the structure of **4** is 3-(hydroxydecanoyl)-(L-Gln)$_4$-didemnin B (Scheme 3).

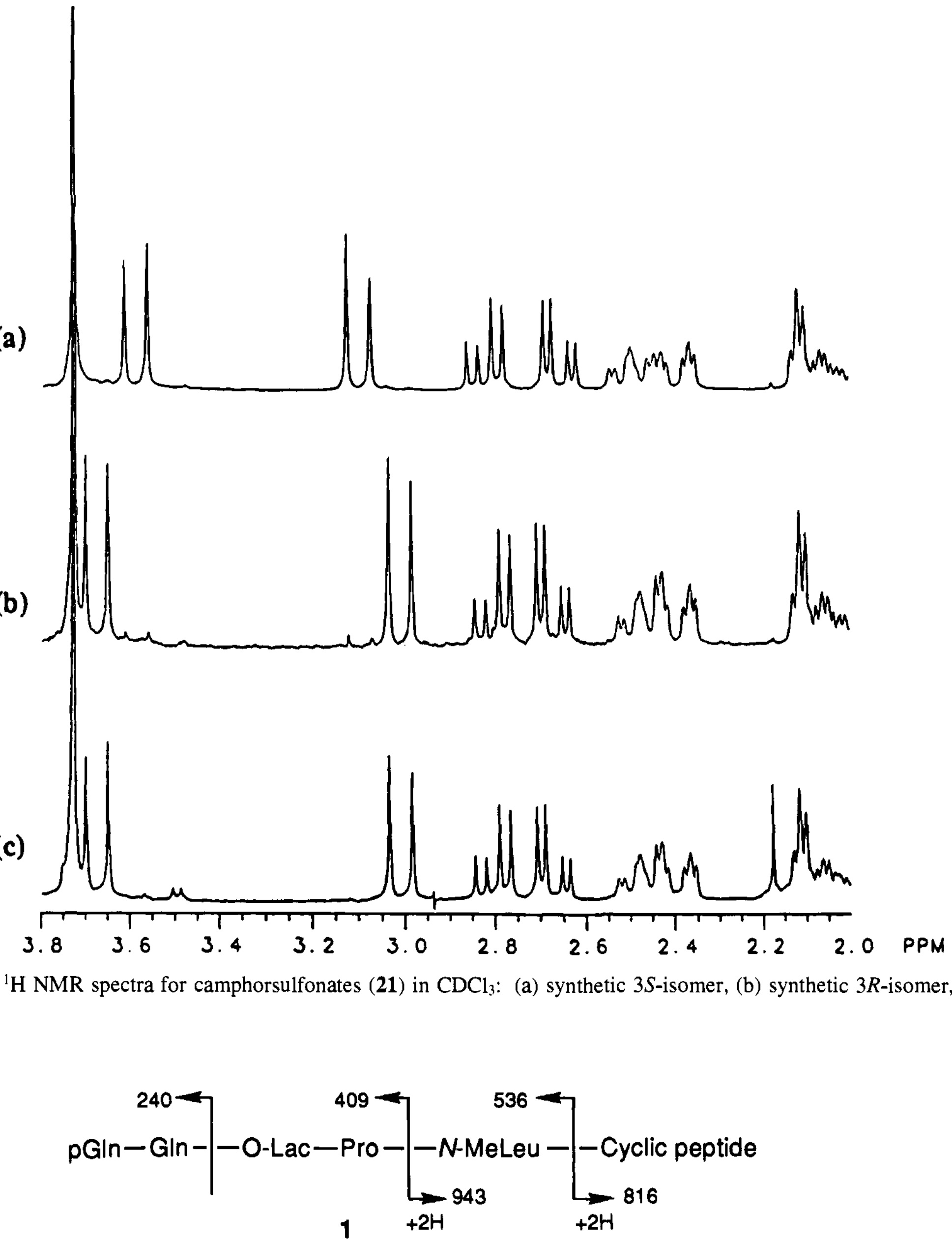
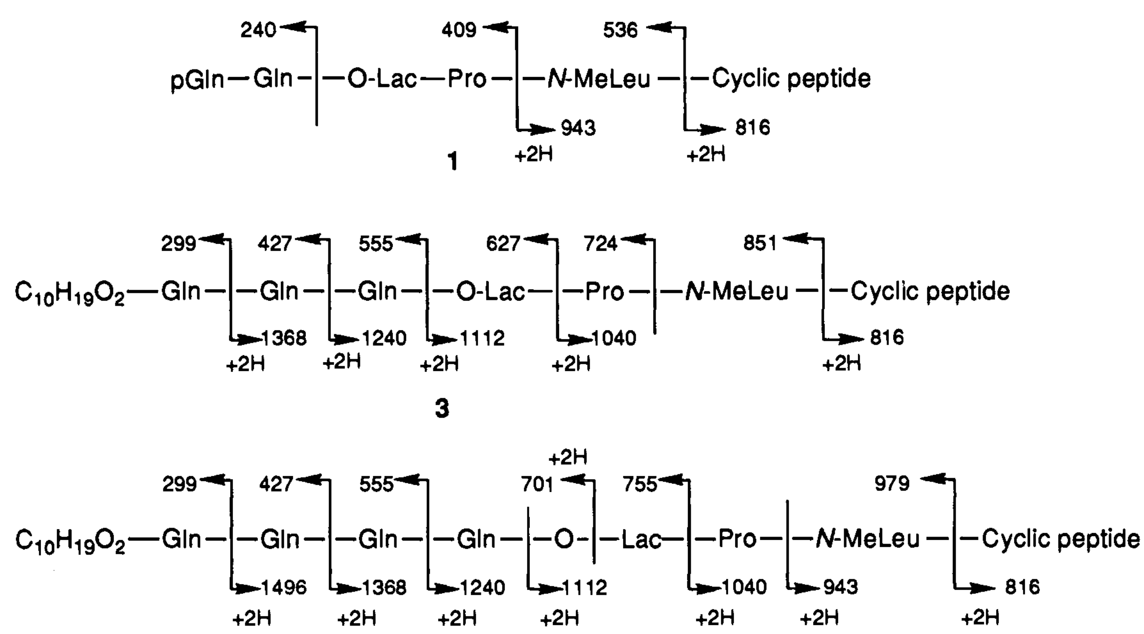


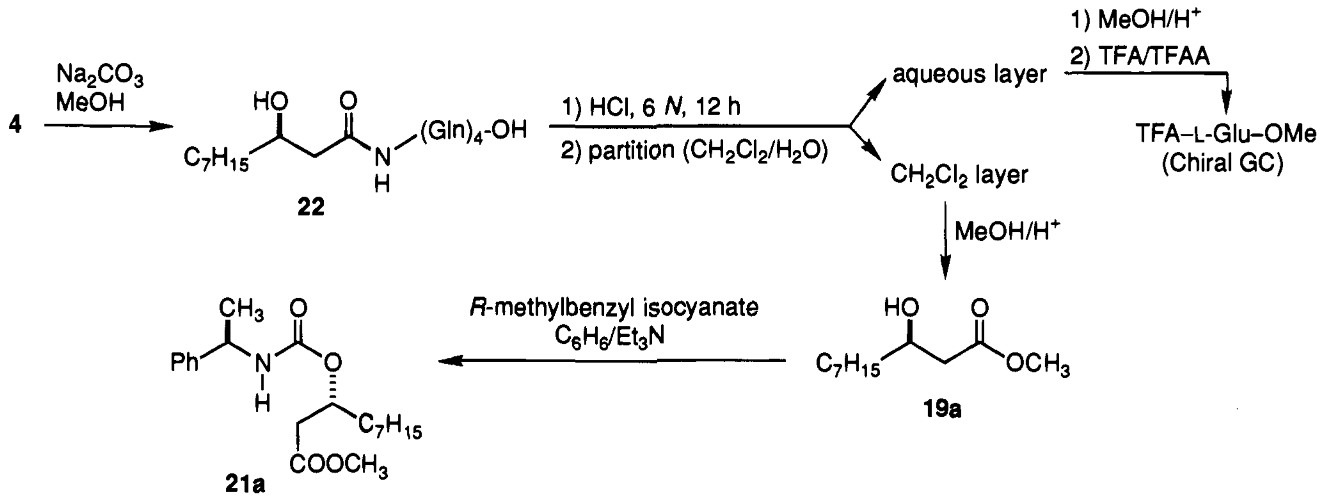
Figure 2. 300-MHz ^{1}H NMR spectra for camphorsulfonates (**21**) in $CDCl_3$: (a) synthetic 3*S*-isomer, (b) synthetic 3*R*-isomer, and (c) natural 3*R* derivative.

Scheme 3

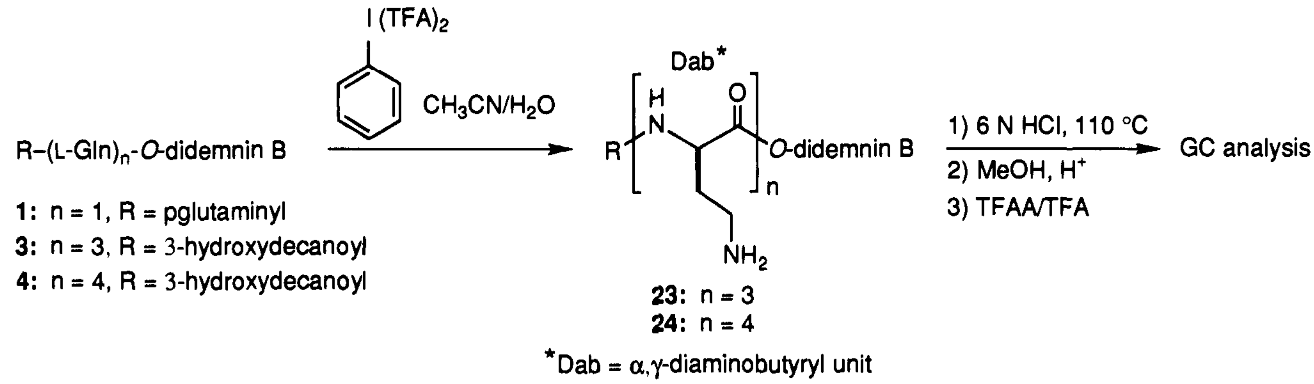


The stereochemistry of the 3-hydroxydecanoyl group in **4** was also determined as *R* as shown in Scheme 4. Basic hydrolysis of **4** gave the side chain compound **22** as a MeOH-insoluble precipitate whose acid hydrolysate showed peaks for 3-hydroxydecanoic acid and L-Glu on chiral GC analysis. The structure of **22** was deduced from its FAB mass spectrum (molecular ion at *m/z* 701) and FABMS/CID/MS data on the molecular ion, which showed sequential losses for ($C_{10}H_{19}O_2$-Gln), Gln, Gln, and Gln-OH. Hydrolysis of **22** followed by methylation gave methyl ester **19a**, which was then treated with

Scheme 4



Scheme 5



(R)-methylbenzyl carbamate to give a reaction product containing mainly **21a** (CIMS m/z 350.2331, Δ 0.1 mmu). HPLC of the reaction product **21a** showed it to coelute with the synthetic R-isomer **21a**, assigning the configuration of the hydroxydecanoyl unit in **4** as the same as in **3**, i.e. R.

Linkages of Glutaminyl Subunits in 3 and 4. The glutaminyl side chain linkages in **3** and **4** were determined by treating them with *I,I*-[bis(trifluoroacetyl)]iodobenzene (BTI) in CH_3CH-H_2O (1:1) to give rearranged products **23** and **24**.[16] The structures were confirmed by HRFABMS data for both **23** (1582.9702, $C_{79}H_{132}N_{13}O_{20}$, Δ 1.0 mmu, M + H) and **24** (1684.0431, $C_{83}H_{141}N_{15}O_{21}$, Δ −0.5 mmu, M + H), along with the two IR absorptions near 3400 and 3150 cm^{-1} (NH_2), to be (3-hydroxydecanoyl)-(α,γ-diaminobutyryl)$_3$-didemnin B and (3-hydroxydecanoyl)-(α,γ-diaminobutyryl)$_4$-didemnin B, respectively (Scheme 5). Separate GC analyses of the derivatized acid hydrolysates of both **23** and **24** showed peaks corresponding to the L-α,γ-diaminobutyryl derivative, indicating an α-linkage for the glutaminyl units of **3** and **4**, as seen in **11** and **12**.[1b]

Structure of Didemnin M (1). The molecular formula of **1** was deduced to be $C_{67}H_{102}N_{10}O_{19}$ on the basis of HRFABMS data (1351.7392, Δ 0.9 mmu, M + H). The ^{1}H NMR spectrum of **1** resembled that of didemnin B, suggesting that they were related. In the ^{13}C NMR spectrum of **1**, peaks for a total of 66 carbons including 12 carbonyls for amides or esters were observed. (One carbonyl peak was overlapped.) The FABMS fragmentation pattern for the side chain and HRFABMS data on each fragmentation ion established the sequence pGlu-Gln-Lac-Pro-MeLeu- (Scheme 3). Chiral GC analysis of the derivatized acid hydrolysate of **1** showed the same components as in **12**. Treatment of **1** with BTI followed by GC analysis assigned an α-linkage to the Gln unit in **1**. The structure of the rest of the molecule was concluded to be the same as **9** since the mild basic hydrolysis product was identical to **9** (HPLC, ^{1}H NMR, and FABMS). All the above data assigned the structure of didemnin M (**1**) as L-pGlu-L-Gln-didemnin B.

Structures of Didemnin N (2) and Nordidemnin N (5). The HRFABMS data for the protonated molecular ion of didemnin N (**2**) at m/z 1084 agreed with the molecular formula $C_{55}H_{86}N_7O_{15}$ (M + H, Δ 0.5 mmu). The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** were similar to those of **9** except for the lack of two methyl signals and the presence of a new amide NH signal at 6.06 ppm. Chiral GC analysis of the hydrolysate of **2** showed the presence of L-Tyr instead of L-Me_2Tyr in **2**, but all other peaks were the same as those of **9**. Sequences of **2** were shown to be the same as those of **9** by FABMS/CID/MS analysis, which is summarized in Table 2. Almost every important fragmentation ion for **2** appeared parallel to one for **9** but differed by 28 mu if the fragment contained tyrosine instead of Me_2Tyr.

Nordidemnin N (**5**) showed ^{1}H NMR characteristics similar to those of **2**, but the molecular weight of **5** (1070.5996, M + H) agreed with a protonated molecular formula of $C_{54}H_{84}N_7O_{15}$, which was 14 mu smaller than **2**. Comparison of GC data for the derivatized acid hydrolysate of **5** with those for nordidemnin B (**14**) showed the presence of a (4R)-amino-(3S)-hydroxy-5-methylhexanoyl (=norstatine, Norsta)[1a,c] residue in **5**. FABMS/CID/MS of **5** showed fragmentation ions almost parallel to those of **2** but differing by 14 mu if the fragment contained the Norsta unit (Table 2). These data constructed the same sequence in **5** as in **2** and assigned the structures of **2** and **5** to be [Tyr[5]]-

Table 1. ^{1}H and ^{13}C NMR Data[a] for Didemnins B (**9**) and N (**2**) and ^{1}H NMR Data for Didemnin A (**8**) and Epididemnin A_1 (**6**) in $CDCl_3$

		didemnin B (**9**)		didemnin N (**2**)		didemnin A (**8**)		epididemnin A_1 (**6**)	
subunit	position	^{13}C (δ)	^{1}H [δ (ppm), mult *J* (Hz)]	^{13}C (δ)	^{1}H [δ (ppm), mult *J* (Hz)]	^{13}C (δ)	^{1}H [δ (ppm), multi *J* (Hz)]	^{13}C (δ)	^{1}H [δ (ppm), mult *J* (Hz)]
Ist[1]	α	38.7	2.61, dd, 17.0, 10.5	38.7	2.55, dd, 10.0, 17.7	38.5	2.51, m	37.1	2.51, br d, 15.0
			3.24, d, 17.0		3.21, d, 17.5		3.06, d, 17.5, 1.0		2.88, br m
	β	67.8	4.03, dt, 9.0, 4.0	67.3	4.03, m	67.6	3.96, t, 9.5	68.8	4.30, br s
	γ	55.3	4.08, dt, 8.5, 4.0		4.03, m	55.5	4.06, dt, 9.5, 4.3		4.19, m
	N*H*		7.17, d, 10.0		7.13, d (9.0)		7.72, d, 9.5		8.16, d, 9.0
Hip[2]	α	49.4	4.22, q, 7.0	49.0	4.21, m	49.4	4.14, q, 7.0	48.7	3.80, q, 7.0
	α-CH_3	15.2	1.29, d, 7.0	15.2	1.30, d, 7.0	15.5	1.33, d, 7.0	15.2	1.29, d, 7.0
	β			205.0		205.1		204.9	
	γ	81.3	5.15, d, 3.0	81.1	5.18, d, 3.5	81.5	5.70, d, 3.5	83.1	5.10, d, 7.0
	δ			31.1		31.1		30.1	
Leu[3]	α	49.4	4.78, ddd, 11.5, 9.5, 1.5	49.3	4.75, m	49.8	4.77, ddd, 9.5, 7.5, 1.5	50.8	4.93, br t, 10.5
	β	41.3	1.20, m	41.5	1.15, m	41.3	1.38, m	40.6	1.25, m
					1.30, m		1.58, m		
	N*H*		7.79, d, 9.5		7.92, d, 9.0		8.22, d, 9.5		7.93, d, 10.0
Pro[4]	α	57.1	4.61, dd, 8.0, 5.0	60.6	4.75, m	57.4	4.59, dd, 5.5, 8.3	56.9	4.73, dd, 8.5, 6.0
	β,γ	27.8	1.79, m	28.3	1.98, m	27.9	1.76, m	29.7	1.87, m
			2.11, m		2.25, m		2.12, m		2.16, m
	δ	46.8	3.56, m	47.1	3.58, m	47.2	3.58, m	47.0	3.65, m
			3.65, m		3.66, m		3.69, m		3.79, m
Me_2Tyr[5]	α	66.3	3.56, m	54.5	4.35, m	66.2	3.57, dd, 4.5, 10.5	66.4	3.61, dd, 11.0, 4.0
	β	33.8	3.14, dd, 14.0, 11.5	34.9	3.10 (2H), m	34.0	3.16, dd, 14.5, 10.5	33.8	3.15, dd, 15.0, 11.0
			3.35, dd, 14.0, 4.0				3.35, dd, 14.5, 4.5		3.30, dd, 15.0, 4.5
	NCH_3	38.6	2.53, s			35.2	2.54, s	35.5	2.67, s
	OCH_3	55.1	3.76, s			55.3	3.78, s	55.3	3.81, s
	N*H*				6.06, d, 8.0				
Thr[6]	α	57.6	4.50, dd, 5.0, 2.0	57.4	4.63, dd, 7.0, 2.0	54.8	4.84, dd, 9.5, 3.5	*b*	4.54, br dd, 7.3, 3.8
	β	70.3	5.39, dd, 6.0, 2.0	71.5	5.08, br q, 6.5	71.1	5.01, dq, 6.0, 3.5	70.9	5.01, dq, 6.5, 3.5
	γ	16.8	1.35, d, 6.5	16.7	1.37, d, 6.5	16.7	1.30, d, 6.0	*b*	1.41, d, 6.0
	N*H*		7.66, d, 5.0		7.59, d, 6.5		7.72, d, 95		7.87, br s
MeLeu[7]	α	55.1	5.34, dd, 11.5, 4.0	54.9	5.38, dd, 4.0, 11.5	62.9	2.92, dd, 8.0, 6.0	*b*	2.88, m
	β					42.3	1.39, m	*b*	*b*
							1.57, m		
	NCH_3	38.6	3.12, s	31.1	3.13, s	38.7	2.52, s	38.6	2.36, s
Pro[8]	α	56.6	4.71, t, 7.5	56.9	4.21, m				
	β	28.3	1.95, m	27.5	1.98, m				
			2.19, m		2.10, m				
	δ	46.9	3.56, m	47.2	3.44, m				
			3.65, m		3.66, m				
Lac[9]	α	65.9	4.37, m	65.9	4.32, q, 7.0				
	CH_3	20.1	1.35, q, 6.5	20.1	1.39, d, 6.5				

[a] Assignments were based on homo- and heteronuclear COSY, APT, and HMQC data. [b] Missing, probably due to peak broadening.

Table 2. FABMS/MS Peaks for Didemnins B (**9**) and N (**2**) and Nordidemnin N (**5**)

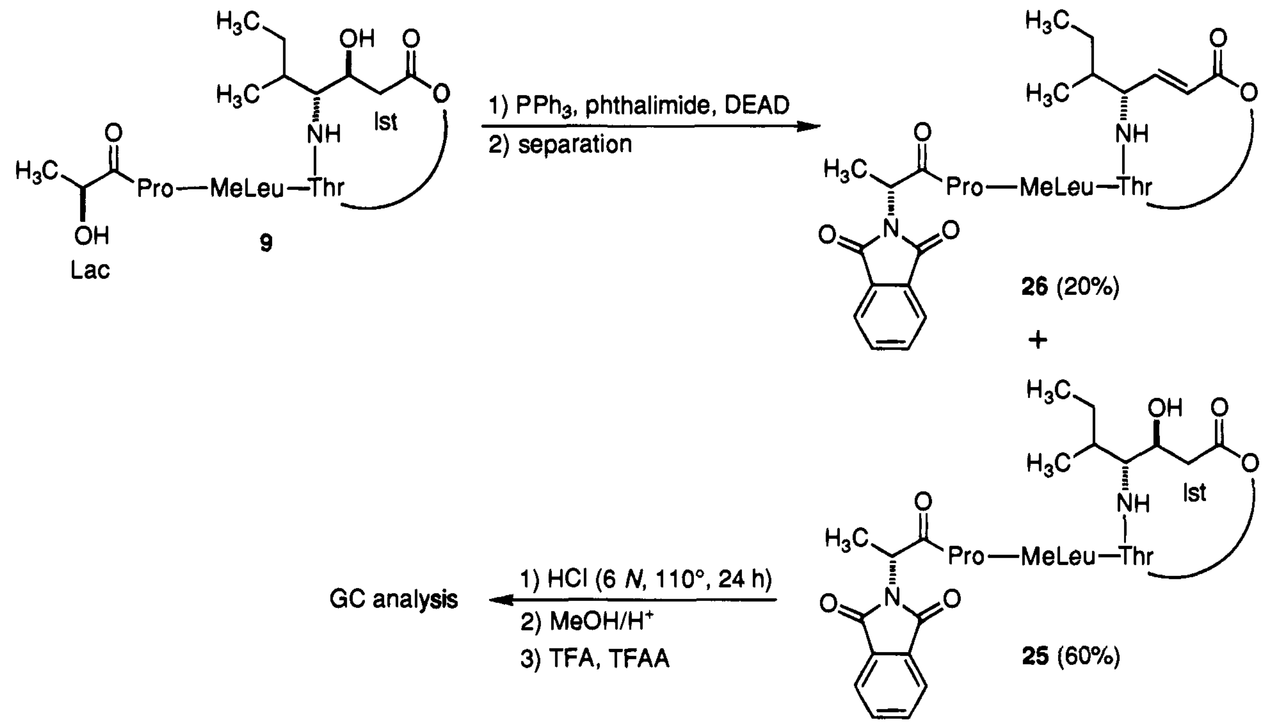
obsd ion (rel intens, %)			
9	**2**	**5**	ion species (positive)
1112 (100)	1084 (100)	1070 (100)	M + H
1094 (2)	1066 (20)	1052 (23)	M + H − H_2O
1084 (100)			M + H − CO?
	1049 (0.6)	1035 (6)	
1040 (1.3)	1012 (1.3)	998 (1.5)	M + H − [Lac]
943 (1.1)	915 (1.8)	901 (2)	M + H − [Lac-Pro]
861 (0.8)	833[a] (0.7)	819[a] (0.7)	M + H − [Leu-Hip] + H_2O[b]
843 (0.5)	815 (0.9)	801 (1.0)	M + H − [Leu-Hip]?
816 (3)	788 (1.8)	774 (1.6)	M + H − [Lac-Pro-MeLeu]
686 (0.1)	658[a] (0.4)	658[a] (0.6)	M + H − [Ist(norSta)-Hip-Leu]
537 (0.2)	537[a] (0.2)	523 (1.0)	[Lac-Pro-MeLeu-Thr-Ist(norSta)] + H − H_2O
398 (0.3)	398 (0.1)	398 (0.2)	[Lac-Pro-MeLeu-Thr] + H?
375 (1.1)	347 (0.3)	347 (0.8)	
307 (5.5)	279 (3.4)	279 (4.5)	[Tyr(Me_2Tyr)-Pro-CO-] + H?
297 (33)	297 (55)	297 (55)	[Lac-Pro-MeLeu] + H
170 (50)	170 (45)	170 (55)	[Lac-Pro] + H
142 (40)	142 (20)	142 (45)	[Lac-Pro] + H − CO

[a] HRFAB data were obtained. [b] For assignment, see ref 1c.

didemnin B and [Tyr^5]nordidemnin B, respectively. The configurations of the Hip, MeLeu, and Lac units, undetermined by the chiral CG, are assigned below.

Configuration of the Lactyl Subunit of Didemnin N (2). In the case of **9**, no trace of lactic acid was detected by GC or GC/MS analysis of the acid hydrolysate,[1c] probably due to decomposition or evaporation of lactic acid during the hydrolysis−derivatization process. Chemical methods such as the modified Horeau's method[17] were attempted to determine the configuration of the Lac moiety in **9**, but limited amounts of sample hampered unambiguous assignments. The configuration was established ultimately by semisynthesis of **9**.[1c,3a] The

Scheme 6



capillary chiral GC method using a Chirasil Val-III fused silica stationary phase was not successful in the separation of enantiomeric pairs of simple α-hydroxy esters as acyl derivatives such as the trifluoroacetate, but separations of diastereomeric derivatives—carbamate derivatives or the 3-hydroxypentanoate—have been reported.[18]

In the present study, a general and relatively easy method for the determination of the absolute configuration of α-hydroxy acids using a combination of the Mitsunobu reaction[19] and chiral GC was developed and the stereochemistry of the lactyl unit in **2** was determined unambiguously. The method involved stereoinversion during replacement of the α-hydroxyl group by phthalimide followed by standard acid hydrolysis and derivatization. Treatment of **9** with 5 equiv of triphenylphosphine, diethyl azodicarboxylate (DEAD), and phthalimide in THF gave two Mitsunobu inversion products, **25** (60%) and **26** (20%), after separation by a silica gel column (Scheme 6). Structures of both products were assured by their spectral data. The molecular formula for **25** ($C_{65}H_{93}N_8O_{16}$, M + H), the major product, was secured by HRFABMS data (*m/z* 1241.6710). The difference in the molecular formula between **25** and **9**, C_8H_3-NO, corresponds to that of phthalimide − H_2O. In the ^{1}H NMR spectrum, the α-proton of the Lac unit (quartet at 4.98 ppm) was shifted from its position in **9** (multiplet, 4.38 ppm) and new aromatic signals were observed between 7.7 and 7.8 ppm (4 H). These data indicated that the hydroxyl group of the Lac unit was replaced by a phthalimide group. On the other hand, the FABMS spectrum of the byproduct **26** showed a molecular weight 18 mu lower than that of **25**. HRFABMS data (1223.6632) agreed with the formula of **26** ($C_{65}H_{91}N_8O_{15}$, Δ 2.8 mmu), which corresponded to the molecular weight of **25** − H_2O. ^{1}H NMR data for **26** showed a new pair of double doublets at 6.72 and 6.30 ppm coupled to each other by 15.5 Hz, indicating dehydration occurred at the β-OH of the Ist moiety to give the *trans* elimination product.

Compound **25** was hydrolyzed and the hydrolyzate derivatized ((TFA)OMe) and analyzed on chiral GC (Chirasil Val-III). A peak for (trifluoroacetyl)-D-alanine methyl ester was identified on GC by coinjection with an authentic racemic sample, which agreed with the original L-configuration of the Lac unit in **9**. The Mitsunobu reaction proceeds almost exclusively by S_N2 inversion, and the side reaction is β-elimination in the case of β-keto alcohols.[20] This side reaction occurred at the Ist unit, giving the byproduct **26**, whose β-hydroxyl group was eliminated to give the *trans* olefin. The lactyl hydroxyl group was also replaced by phthalimide in **26**.

A similar method was used to assign the Lac configuration in **2**. Compound **2** was treated with 10 equiv of each reagent, and the product was separated, hydrolyzed with acid, and derivatized. GC analysis of the resulting product showed a peak for D-Ala, assigning the configuration of the lactyl unit in **2** as *S* (L-Lac) (Figure 3). Assignments of the configurations of the Hip and MeLeu units in **2** are discussed below, together with the structure determination of epididemnin A_1 (**6**).

Structure of Epididemnin A_1 (6). Epididemnin A_1 (**6**) is an isomer of didemnin A (**8**) which has the same molecular weight and gives very similar FABMS/CID/MS spectra and chiral GC data. Since they give practically the same FABMS and FABMS/CID/MS data, their sequences are concluded to be the same. Thus, the new compound must either be a conformer or differ in the stereochemistry of the Hip, Me_2Tyr,

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(18) For enantiomeric hydroxy acids separation on GC, see: (a) Slessor, K. N.; King, G. G. S.; Miller, D. R.; Winston, M. L.; Cutforth, T. L. *J. Chem. Ecol.* **1985**, *11*, 1659−1667. (b) König, W. A.; Benecke, I.; Lucht, N.; Schmidt, E.; Schulze, J.; Sievers, S. *J. Chromatogr.* **1983**, *279*, 555−564. (c) König, W. A.; Francke, W.; Benecke, I. *J. Chromatogr.* **1982**, *239*, 227−231. (d) König, W. A.; Benecke, I.; Sievers, S. *J. Chromatogr.* **1981**, *217*, 71−79. (e) König, W. A.; Benecke, I.; Sievers, S. *J. Chromatogr.* **1982**, *238*, 427−432. (f) Koppenhoefer, B.; Allmendinger, H.; Nicholson, G. J.; Bayer, E. *J. Chromatogr.* **1983**, *260*, 63−73.

(19) (a) Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1972**, *94*, 679−680. (b) Mitsunobu, O. *Synthesis* **1981**, 1−28.

(20) Wada, M.; Sano, T.; Mitsunobu, O. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 2833−2835.

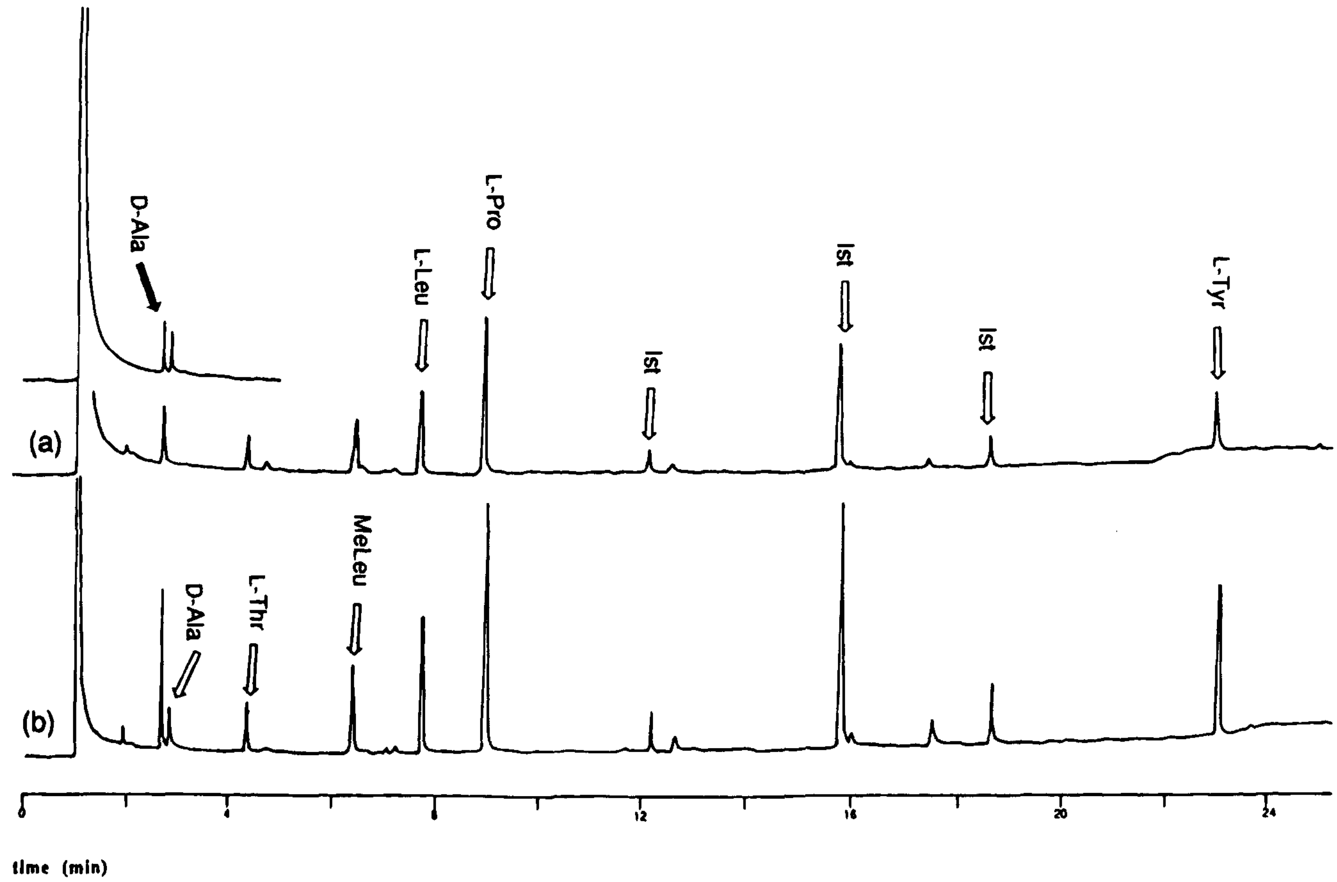

Figure 3. GC analysis of precolumn derivatized acid hydrolysates of (a) an imide obtained from the Mitsunobu reaction of didemnin N (**13**) and (b) a mixture of the above and *N*-(trifluoroacetyl)-D,L-alanine methyl ester.

or MeLeu subunit, whose stereochemistry cannot be determined by the chiral GC experiments.

The configurations of the D-MeLeu and L-Me_2Tyr units were assigned by the chiral TLC method.[21] The two amino acids were isolated from the acid hydrolysate of **6** by C-18 HPLC, and each amino acid was compared by chiral TLC with authentic samples. Similar analysis assigned the D-configuration of the MeLeu unit in **2**.

The above results indicated that **6** must be either a conformer or a Hip epimer of **8**. In the ^{1}H NMR data for **6**, significant differences in chemical shifts and coupling constants from those of **8** (didemnin A) were observed for the Thr-Ist-Hip region (Table 1). The coupling constant ($J_{4,5}$ = 7 Hz) of the H-4 doublet in Hip is larger than the typical value (3.5 Hz) commonly observed in other didemnins, suggesting a difference in the stereochemistry of the Hip unit in **6**, which had earlier been established as 2*S*,4*S*.[22] Since the amount of the sample was limited, an analytical method was developed using chiral capillary GC in order to determine the stereochemistry of the Hip unit in **6**.

The basis of the strategy is the chiral GC analysis of a cyclized dihydro Hip derivative, 3,4-dihydro-5-isopropyl-3-methyltetronic acid, designated as "γ-lactone A", from acid hydrolysates of reduced didemnins.[1c] Didemnin A (**8**) was converted to [2,3-dihydro-(2*S*,3*R*,4*S*)-Hip^2]didemnin A (**27**) with $NaBH_4$ in THF$-H_2O$ to avoid racemization at the α-position of the Hip unit during acidic hydrolysis. Compound **27** was hydrolyzed (6 N HCl, 85 °C, 12 h), and the product was derivatized ((TFA)OMe) and analyzed by GC (Scheme 7).

In the above conversion, the TFA ester of the γ-lactone was obtained with retention of the original stereochemistry at C-2 and C-4 of Hip. Standard samples of the four stereoisomers of γ-lactone A were prepared by reduction of the keto group of (4*S*)-*O*-benzyl-Hip ethyl ester (**28**)[22] with $NaBH_4$ (Scheme 8). The product, a diastereomeric mixture of 4-*O*-benzyl diols (**29a**$-$**32a**) with defined configuration at C-4 (*S*), was separated by HPLC, then deprotected and cyclized to give four stereoisomers of γ-lactone A, **29**$-$**32**, which were converted to the trifluoroacetates **29b**$-$**32b**. GC analysis using a Chirasil Val-III column gave the respective four peaks for compounds **29b**$-$**32b**, listed in order of increasing retention times.

Stereochemistries at C-2 and C-3 for **29**$-$**32** were assigned as follows. The ^{1}H NMR data for **30** were essentially the same as for (2*R*,3*R*,4*R*)-γ-lactone A, an antipode of **30** reported by Joullié and co-workers whose absolute stereochemistry was determined by X-ray crystallography.[23] The physical data for **30** were also identical to reported values except for the opposite sign of the specific rotation. Thus, stereochemistry for **30** was assigned to be (2*S*,3*S*,4*S*). The stereochemistries of acetates **29** and **32** had been assigned, based on the ^{1}H NMR coupling constants and chemical reactivities, by Joullié and co-workers.[23] In the present study these assignments were confirmed by ^{1}H and ^{13}C NMR data compared to data for aldono-1,4-lactones **33**$-$**36**.[24] Coupling constants $J_{2,3}$ and $J_{3,4}$ of **29**, **31**, and **32** showed patterns similar to those of 33, 35, and 36, respectively. However, the coupling constants $J_{2,3}$ and $J_{3,4}$ for **30** were very different from those reported for the corresponding sugar lactone **34** (Table 3).[25] Although this may be due to a difference in

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(22) Nagarajan, S. Ph.D. Thesis, University of Illinois, Urbana, IL, 1984.

(23) Ewing, W. R.; Bhat, K. L.; Joullié, M. M. *Tetrahedron* **1986**, *42*, 5863$-$5868.

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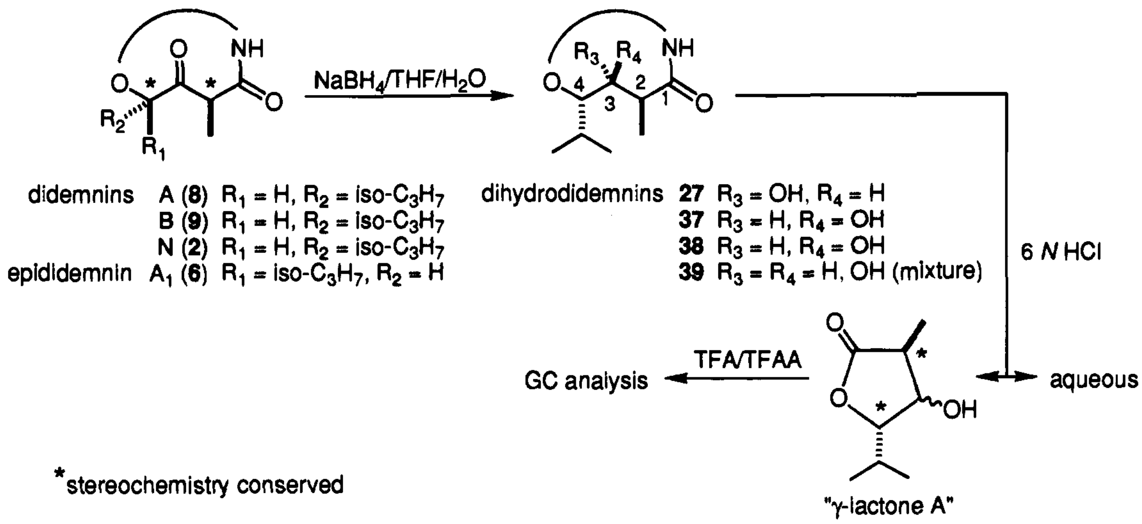
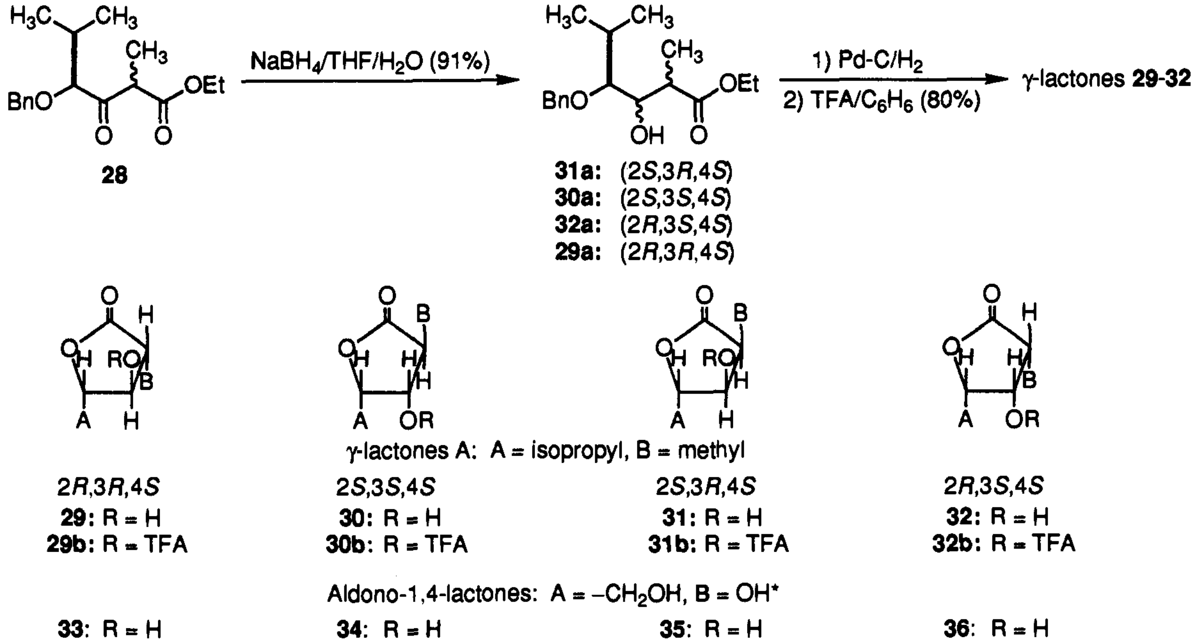
(25) Jaime, C.; Ortuño, R. M.; Font, J. *J. Org. Chem.* **1986**, *51*, 3946$-$3951.

Scheme 7



Scheme 8



*Structures **33-36** are drawn as the L-configuration for comparison to **29-32**. Actual lactones[23] were from D-aldoses.

solvents or in solution conformations[25] between **30** and **34**, the anomaly warrants further study. The ^{13}C chemical shifts at C-2 and C-3 of compounds **29**–**32** followed tendencies similar to those of **33**–**36**.[24a] Specifically, 2,3-*threo* compounds (**29**, **30**; **33**, **34**) showed chemical shifts for C-2 and C-3 downfield by 2-4 ppm from C-2 and C-3 in the 2,3-*erythro* isomers (Table 3). The above data allowed us to assign the stereochemistries at C-2, C-3, and C-4 of γ-lactones A, **29**–**32**, as (2*R*,3*R*,4*S*), (2*S*,3*S*,4*S*), (2*S*,3*R*,4*S*), and (2*R*,3*S*,4*S*), respectively. $^{1}H-^{1}H$ NOE difference spectra for compounds **30**–**32** were also in complete agreement with these assignments.[26] The TFA ester of each lactone A was injected on Chirasil Val-III for GC. These four isomers had retention times distinguishable from one another, with the elution order being **29** < **30** < **31** < **32** (Table 3).

For the GC analyses of γ-lactones A derived from didemnins, didemnins A (**8**), B (**9**), and N (**2**) and epididemnin A_1 (**6**) were first treated with $NaBH_4$ separately to give dihydrodidemnins **27**, **37**, **38**, and **39**, respectively. Each of these compounds was hydrolyzed and derivatized as shown in Scheme 7. The γ-lactone A from **27** (dihydro A) gave only one dominant peak, coeluting with synthetic **31**. The γ-lactones A derived from **37** and **38** (dihydro B and N) showed a different dominant peak, coeluting with synthetic **30**. These data not only agreed with the previous assignments of the C-2 and C-4 relative stereochemistry of Hip in didemnins A and B[3a,22] but also determined that of didemnin N (**2**) to be (2*S**,4*S**). In the case of dihydroepididemnin A_1 (**39**, [4-epiH_2Hip[2]]didemnin A), however, the same GC derivative gave two peaks coeluting with the first and fourth peaks, **29b** and **32b**. These results can be summarized (i) that γ-lactones from **27**, **37**, and **38** coeluted with synthetic **31b**, **30b**, and **30b**, respectively, having (2*S*,4*S*) relative stereochemistry and (ii) that didemnin A- and didemnin

(26) The following NOE's were observed. **30**: 2-CH_3 (irradiated) → 3-H, 4-H (enhanced). **31**: 2-H ↔ 3-H. **32**: 4-H → 2-H, 3-H; 3-H → 2-H. Due to signal overlap, NOE's of **29** were not measured.

Table 3. Physical Properties of γ-Lactones A (**29–32**) and γ-Aldonolactones (**33–36**)

	γ-lactones A: $R_1 = C_3H_7$, $R_2 = CH_3$			
	29	**30**	**31**	**32**
		^{1}H NMR Coupling Constants (Hz)[a]		
$J_{2,3}$	9.0	0.0	6.5	4.4
$J_{3,4}$	8.0	3.0	1.5	2.7
		^{13}C NMR Data ($CDCl_3$, ppm)		
C-1	missing	179.08	178.32	175.43
C-2	44.26	46.16	39.35	42.53
C-3	73.44	74.64	71.38	71.09
C-4	87.85	87.82	91.23	88.33
C-5	31.03	27.19	30.58	27.06
5-CH_3	18.11	19.96	18.44	19.89
5-CH_3	18.06	18.02	18.29	17.53
2-CH_3	12.62	13.42	8.34	7.97
GC retn time (min)	4.7	6.9	7.6	9.1
mp, °C (lit.[22])	66 (63–65)	118 (120–121)	81–82	108 (109–111)
$[\alpha]_D^{23}$ [a] in $CHCl_3$	−51.5/0.07	−88.3/0.25	−21.2/0.13	−52.7/0.27
(lit.[c] $[\alpha]^{22}{}_D$)[26]	(−45.7/1.13)	(+89.7/0.75)	(−47.0/1.29)	
	D-γ-aldonolactones: $R_1 = CH_2OH$, $R_2 = OH$[b]			
	33	**34**	**35**	**36**
		^{1}H NMR Coupling Constants (Hz)[c]		
$J_{2,3}$	8.7	7.3	5.7	4.8
$J_{3,4}$	8.0	7.3	0.8	2.9
		^{13}C NMR Chemical Shifts (D_2O, ppm)[d]		
C-1	177.05	178.1	179.5	179.1
C-2	74.7	74.1	70.4	71.5
C-3	73.4	73.1	69.9	70.5
C-4	82.2	81.4	87.7	82.5
C-5	60.3	59.9	61.5	60.7

[a] In $CDCl_3$ except for **30** (C_6D_6 due to overlap in $CDCl_3$). [b] Structures were drawn as L for convenience. [c] Reference 24b, in CD_3OD except for **33**, $(CD_3)_2SO$. [d] Reference 24a.

B-type peptides gave opposite stereochemistries at the C-3 positions upon $NaBH_4$ reduction. The latter result suggests a different conformation at Hip in didemnins B and N relative to that in didemnin A. On the other hand, **6** gave two lactones A corresponding to synthetic **29b** and **32b** (2*R*,4*S* derivatives). Thus, the stereochemistry for the Hip subunit in **6** must be (2*R*,4*S*) or its antipode. The assignment of the absolute configuration was carried out unambiguously as follows.

An authentic mixture of eight stereoisomers of γ-lactone A was prepared from racemic *O*-benzyl-Hip ethyl ester by following a similar sequence of reduction, deprotection, cyclization, and precolumn derivatization, carried out without separation of diastereomers. This racemic mixture of the eight stereoisomers of γ-lactone A showed six peaks on the Chirasil Val-III column. Only the enantiomeric pairs for 2,4-*threo*-lactones were separated in this experiment; that is, antipodes in the 2,4-*erythro* lactone series, in the first and sixth peaks, were not separated, but those in the 2,4-*threo* lactone series, (2*S*,3*S*,4*S*)-, (2*R*,3*R*,4*R*)-, and (2*S*,3*R*,4*S*)-, (2*R*,3*S*,4*R*)-γ-lactones, the second, third, fourth, and fifth peaks, respectively, were separated. This result automatically assigned the absolute stereochemistry (2*S*,4*S*) for Hip in **8**, **9**, and the previously unknown didemnin N (**2**), since γ-lactones from those compounds coeluted with the fourth, the second, and the second peaks, respectively. Although Chirasil Val-III did not separate the enantio pairs for 2,4-*erythro*-lactones, a cyclodextrin-fused silica capillary GC column[27] separated those enantiomers very well, giving four pairs of peaks. Coinjection of a mixture of eight stereoisomers of racemic Hip with a mixture of four stereoisomers of (4*S*)-Hip showed that the 4*S*-isomers have longer retention times than the 4*R* isomers for the first and the fourth pairs. The natural γ-lactone A derived from epididemnin A_1 (**6**) coeluted with the faster peaks of the first and the fourth pairs (the first and the seventh peaks) of a racemic mixture of Hip, clearly assigning the configuration of Hip in **6** to be 2*S*,4*R*.

Structure of Acyclodidemnin A (7). The molecular formula of **7** ($C_{49}H_{80}N_6O_{13}$) was assigned on the basis of HRFABMS data for the molecular ion at *m/z* 961 (M + H). This corresponds to the molecular formula of **8** ($C_{49}H_{78}N_6O_{12}$) plus 1 mol of H_2O, suggesting that **7** is a hydrolyzed form of **8**. Since the ^{1}H NMR spectrum of this compound was not well resolved and was not suitable for further interpretation, the structure was assigned by mass spectrometry. Acetylation of **7** gave triacetyl **7** (**7a**). FABMS/CID/MS spectra of **7a** showed major fragmentation ions at *m/z* 752 (M − $HOMe_2Tyr$), 655 (M − HO-Me_2Tyr-Pro), 307 (HO-Me_2Tyr-Pro + $2H^+$), and 210 (HO-Me_2Tyr + $2H^+$), suggesting that Me_2Tyr-OH is a C-terminus of **7**. FABMS/CID/MS data for **7** also showed dominant fragmentation of [Me_2Tyr-Pro]. All the above data supported assigning **7** to be a ring-opened form of **8** in which the ester linkage between Thr and Me_2Tyr has opened to give the linear peptide.

Discussion

The structures of seven new didemnins (six didemnins and acyclodidemnin A) have been determined, as shown in Chart 1. Epididemnin A_1 (**6**), a C-4 epimer of the Hip residue, could be considered to be a product of epimerization at the C-4 position α to the carbonyl group during isolation. However, separate treatment of **8** and **6** with silica gel (in $CHCl_3$−MeOH, 48 h) did not show any interconversion, supporting the argument that **6** is a biosynthetic product. We have previously described synthetic epididemnin A containing [2*R*,4*S*-Hip^2]DA as a

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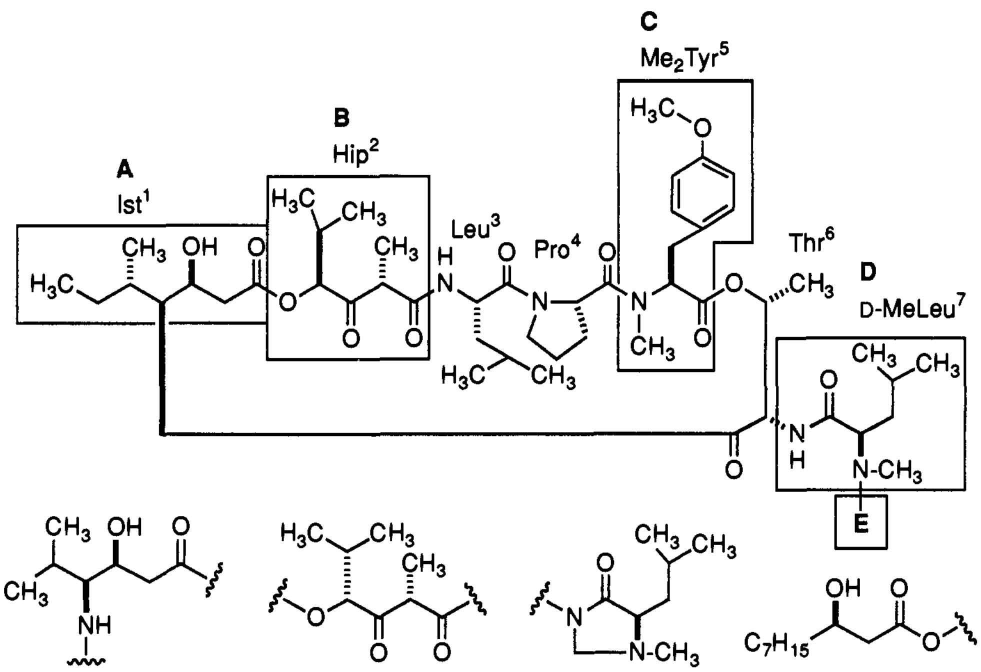
Chart 1



Norsta EpiHip X Hydec

		A	B	C	D	E
Didemnins A	(8)	Ist	Hip	Me_2Tyr	D-MeLeu	-H
B	(9)	Ist	Hiip	Me_2Tyr	D-MeLeu	-Pro-Lac
C	(10)	Ist	Hip	Me_2Tyr	D-MeLeu	-Lac
D	(11)	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-pGlu
E	(12)	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-pGlu
G	(16)	Ist	Hip	Me_2Tyr	D-MeLeu	-CHO
M	(1)	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-pGlu
N	(2)	Ist	Hip	Tyr	D-MeLeu	-Pro-Lac
X	(3)	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-Hydec
Y	(4)	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-Gln-Hydec
Nordidemnins A	(13)	Norsta	Hip	Me_2Tyr	D-MeLeu	-H
B	(14)	Norsta	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac
N	(5)	Norsta	Hip	Tyr	D-MeLeu	-Pro-Lac
Methylenedidemnin A	(15)	Ist	Hip	Me_2Tyr	X	-H
Epididemnin A_1	(6)	Ist	epiHip	Me_2Tyr	D-MeLeu	-H
Acyclodidemnin A	(7)	Ist	Hip	Me_2TyrOH	D-MeLeu	-H

byproduct of the total synthesis of didemnin A.[3a] This compound, an epimer at the α-position of Hip, was converted to didemnin A during the chromatographic process.

Acyclodidemnin A (**7**) could be a partial hydrolyzate of **8** formed during storage or isolation. However, despite the fact that treatment of **8** with 1 equiv of base exclusively hydrolyzed the ester linkage between Ist and Hip, no acyclo compound corresponding to that base treatment product was detected in the extract. Therefore, **7** can also be considered as a natural product—either an enzymatic degradation product or a biosynthetic precursor of **8**.

New didemnins isolated in this study have the following structural variations: in the length and polarity of peptide side chain (didemnins X, Y, and M); in the Tyr-derived subunit—*N,O*-dimethyl Tyr vs Tyr (didemnin N and nordidemnin N); in the stereochemistry of the Hip residue (epididemnin A_1); and in the cyclic structure (acyclodidemnin A). Some of these variations contain structural units which are not easily prepared by chemical modification of the most abundant didemnins A (**8**) and B (**9**).

Didemnins M (**1**), N (**2**), X (**3**), Y (**4**), A (**8**), and B (**9**) showed cytotoxicity versus P388 cells, with IC_{50} = 2.0, 50, 2.0, 2.0, 30, and 0.5 ng/mL, respectively. Epididemnin A_1 (**6**) and acyclodidemnin A (**7**) showed much weaker cytotoxicity (IC_{50} 2.0 and 0.2 μg/mL, respectively). More importantly, **1** showed potent immunosuppressive activity in both the in vitro mixed lymphocyte reaction and the in vivo graft-vs-host reaction assay. Additional biological properties for the above compounds[28] and structure–activity relationships for other didemnins[6] are described elsewhere.

Experimental Section

General Procedures. IR and UV spectra were recorded on IR/32 FTIR and Lambda-3 UV/vis spectrophotometers, respectively. NMR spectra were obtained with QE 300 and GN 500 FT NMR spectrometers using either deuteriochloroform ($CDCl_3$), deuteriomethanol (CD_3OD), or a mixture of both as solvents and internal standards [7.26 (^1H) and 77.0 (^{13}C) ppm for $CHCl_3$, 3.30 (^1H) and 49.0 (^{13}C) ppm for CD_3OD or a mixture of CD_3OD-$CDCl_3$]. FABMS spectra and HRFABMS data were recorded on either a ZAB-SE or a 70-SE-4F spectrometer

(28) (a) Rinehart, K. L. U.S. Patent 4,948,791; *Chem. Abstr.* **1991**, *114*, 214413h. (b) Rinehart, K. L. U.S. Patent 5,294,603; *Chem. Abstr.* **1994**, *121*, 887.

operating in the FAB mode using magic bullet matrix.[29] CIMS spectra or HRCIMS data were recorded with the 70SE-4F using methane as a reagent gas. EIMS and HREIMS data were obtained with CH-5 DF and 731 instruments. Collisionally induced tandem MS/MS spectra in the FAB mode (FABMS/CID/MS) were obtained on a 70-SE-4F four-sector tandem mass spectrometer using helium as a collision gas.

Optical rotations were measured with a DIP 360 or a DIP 370 digital polarimeter with an Na lamp (589 nm) using a 5- × 0.35-cm (1.0 mL) cell. Melting points were determined on a microscope melting point apparatus and are not corrected.

Chromatography. Columns were prepared with commercial grade (large pore, 58 μm) silica gel, Kieselgel 60 (70–230 mesh), styrene–divinylbenzene copolymer gel (NS gel), TLC grade silica gel (2–10 μm), or Sephadex LH-20.

Silica gel (4.6 × 250 mm, 5 μm particle size) C-18, cyanopropyl, and phenyl (250 × 4.6 or 10 mm, 5- or 10-μm particle size) columns were used for HPLC. An Ito multilayer coil separator–extractor was used for HSCCC with a no. 10 column (i.d. = 2.6 mm, V = 380 mL) at 600 rpm.

Hydrolysis and precolumn GC analyses were carried out using a gas chromatograph with the following conditions: (A) Chirasil-Val III capillary column (25 m × 0.32 mm), flow rate 1–2 mL/min, programmed oven temperature [90 °C (4 min) → 180 °C (4 °C/min)]; (B) Cyclodex-B cyclodextrin fused silica capillary column[26] (30 m × 0.25 mm), flow rate 1 mL/min, 20:1 split ratio, programmed oven temperature [90 °C (4 min) → 180 °C (2 °C/min)]; (C) Chirasil-Val III capillary column, flow rate 2 mL/min, programmed oven temperature [80 °C (4 min) → 120 °C (4 °C/min). Other conditions used are noted in each section.

Chiralplate was used for chiral TLC with $CH_3OH-H_2O-CH_3CN$ (50:50:200 or 50:50:30).[21]

Extraction and Initial Separation.[2] A sample (189 kg) of *T. solidum* collected by scuba at a depth of −10 to −40 m off the coast of St. George's Cay, Belize, was extracted with ethanol or 2-propanol and separated by solvent partition and silica gel column chromatography. A polar fraction eluted by $MeOH-CHCl_3$ (6:4) was designated as "fraction A" (132 g). A portion (9 g) of fraction A was employed in the present study.

LC/FABMS of Fraction A.[8] Fraction A (0.5 μg) was analyzed by moving belt LC/FABMS using an RP C-18 silica gel microbore column with $MeOH-H_2O-Et_3N-HOAc$ (77:23:0.23:0.10 v/v) at a flow rate of 0.75 μL/min.

Isolation of 3 and 4. Fraction A (9.0 g) was partitioned between the upper and the lower layers of $EtOAc-heptane-MeOH-H_2O$ (7:4:4:3). Each layer was concentrated in vacuo to give a solid (4.5 g each). A portion of the lower layer (1 g) was separated by HSCCC with $toluene-EtOAc-MeOH-H_2O$ (6:7:7:4) using the lower layer as a mobile phase (flow rate 2 mL/min). Fractions (24 mL/fraction × 40) were collected and monitored by TLC ($CHCl_3-MeOH$ (4:1)). Fractions 11, 12–13, 14–16, and 19–29 gave crude **10**, **11**, **4**, and **3**, respectively.

Didemnin Y (4). Crude **4** (120 mg) was separated by a gravity column chromatography (NS gel) with MeOH to give a peptide fraction (49.6 mg) which was purified by repeated HPLC using an amino column with $CHCl_3-MeOH$ (8:1), a silica gel column with $CHCl_3-MeOH$ (3:1), and a C-18 column with $MeOH-H_2O$ (10:1) to give pure **4** (11.3 mg): mp 230–240 °C (dec); $[\alpha]^{20}D$ −65° (c 0.93, $CHCl_3-CH_3OH$ (4:1)); IR (film) 3310, 2950, 1720, 1650 cm^{-1}; UV (CH_3OH) λ_{max} 204 (log ϵ 4.68), 224 sh (4.38), 277 (3.20); ^1H NMR ($CDCl_3-CD_3OD$) δ 7.30 (2 H, d, J = 8.1), 6.70 (2 H, d, J = 8.1), 3.74 (3 H, s), 2.98 (3 H, s), 2.49 (3 H, s); FABMS m/z 1796 (M + H), 1795, 1240, 1112, 1040, 979, 943, 816, 723, 701, 555. Anal. Calcd for $C_{87}H_{140}N_{15}O_{25}$ (M + H): M_r 1795.0145. Found: M_r 1795.0119 (HRFABMS).

Didemnin X (3). Crude **3** (248 mg) was separated on an NS gel column using MeOH, then passed through a short silica gel column (pretreated with ammonia gas) with $CHCl_3-MeOH$ (4:1). Purification by HPLC on a silica gel column with $CHCl_3-MeOH$ (4:1) and a C-18 column with $MeOH-H_2O$ (10:1) gave pure **3** (107 mg): amorphous powder; mp 156–158 °C; $[\alpha]^{20}_D$ −88.6° (c 6.35, $CHCl_3$); IR (film) 3450, 3300, 2950, 1720, 1650 cm^{-1}; UV λ_{max} 204 (log ϵ 4.72), 224 sh (4.51), 277 (3.20); ^1H NMR ($CDCl_3-CD_3OD$) δ 7.02 (2 H, d, J = 8.4), 6.78 (2 H, d, J = 8.4), 3.72 (3 H, s), 3.00 (3 H, s), 2.47 (3 H, s); FABMS m/z 1668 (M + H), 1415, 1368, 1240, 1112, 1040, 943, 851. Anal. Calcd for $C_{82}H_{132}N_{13}O_{23}$ (M + H): M_r 1666.9559. Found: M_r 1666.9533 (HRFABMS).

Methanolysis of 3. A solution of semipure **3** (122 mg) in MeOH (2 mL) was treated with Na_2CO_3 (25 mg) at room temperature for 0.5 h, filtered, and evaporated. The MeOH-soluble residue was purified on HPLC (C-18) with $MeOH-H_2O$ (4:1) to give **9** (18 mg): white powder; mp 152–154 °C (lit.[2a] 152–154 °C); $[\alpha]^{20}_D$ −83.7° (c 0.4, $CHCl_3$) (lit.[2a] $[\alpha]^{25}_D$ −77.5°); TLC and ^1H NMR identical with those of authentic **9**. Anal. Calcd for $C_{57}H_{90}N_7O_{15}$ (M + H): M_r 1112.6495. Found: M_r 1112.6502 (HRFABMS).

The MeOH-insoluble residue was washed with MeOH, dissolved in DMSO, and filtered to give white amorphous **17** (35 mg): $[\alpha]^{20}_D$ −19° (c 0.4, DMSO); FABMS m/z 587, 555, 427, 299, 188. Anal. Calcd for $C_{26}H_{47}N_6O_9$ (M + H): M_r 587.3405. Found: M_r 587.3401 (HRFABMS).

Hydrolysis of 17. A solution of **17** (12.3 mg) was hydrolyzed in HCl (3 N, 1 mL, 120 °C, 8 h). The CH_2Cl_2 layer after workup afforded **18a**, a white powder: ^1H NMR ($CDCl_3$) δ 4.03 (1 H, br s), 2.55 (1 H, br d, J = 17.7), 2.45 (1 H, dd, J = 10.5, 17.1), 1.63–1.38 (2 H, m), 1.25 (br s), 0.88 (2 H, br t, J = 5.7); HREIMS m/z 89.0239 ($C_3H_5O_3^+$). Anal. Calcd for $C_{10}H_{21}O_3$ (M + H): M_r 189.1491. Found: M_r 189.1486 (HRFABMS).

The acid **18a** was treated with a mixture of MeOH–AcCl (9:1, 120 °C, 30 min) and separated (silica gel, $CH_2Cl_2-EtOAc$ (5:1)) to give **19a** (890 μg) as an oil: ^1H NMR ($CDCl_3$) δ 4.00 (1 H, m), 3.71 (3 H, s), 2.46 (1 H, dd, J = 3.3 16.5), 2.40 (1 H, dd, J = 9.0, 16.8), 1.54–1.34 (m), 1.28 (br s), 0.88 (3 H, t, J = 6.3); FABMS m/z 203 (M + H). Anal. Calcd for $C_{11}H_{23}O_3$ (M + H): M_r 203.1647. Found: M_r 203.1646 (HRFABMS).

Racemic Methyl 3-Hydroxydecanoate (19ab).[11] Octanoyl chloride (16.2 g, 0.088 mol) was added to a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid, 11.5 g, 0.080 mol) in CH_2Cl_2 (100 mL) and C_5H_5N (12.8 mL) over 10 min at 0 °C. The reaction was carried out as in the reference to give a β-keto ester intermediate as a light-yellow oil (14.6 g) which was carried out to the next step without further purification. A portion of the oil (650 mg, 3.2 mmol) was dissolved in THF (10 mL), and a solution of $NaBH_4$ (120 mg, 3.2 mmol) in H_2O (1 mL) was added at 0 °C. The reaction mixture was stirred for 1 h and quenched with acetone (5 mL). The CH_2Cl_2 soluble material was purified (silica gel, $CH_2Cl_2-EtOAc$ (3:1)) to give **19ab** as a colorless oil (359 mg, 56%): ^1H NMR identical with that of natural **19a**; ^{13}C NMR δ 177.4, 67.9, 51.6, 41.1, 36.5, 31.7, 29.4, 29.1, 25.4, 22.5, 14.0. Anal. Calcd for $C_{11}H_{22}O_3$: C, 65.01; H, 10.87. Found: C, 65.31; H, 10.96.

Racemic 3-Hydroxydecanoic Acid (18ab). Ester **19ab** (166 mg, 0.82 mmol) was hydrolyzed (6 N NaOH, 0.5 mL, 110 °C, 1 min). The reaction product was dissolved (H_2O, 1 mL), and the solution was neutralized (6 N HCl, 0.5 mL) and extracted (CH_2Cl_2) to give **18ab** (127 mg, 83%) as fine crystals: mp 54–56 °C (lit.[11b] mp 56.6 °C); ^1H NMR identical with that of natural **18**. Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.80; H, 10.71. Found: C, 63.66; H, 10.88.

Conversion of Synthetic Ester 19ab to Its (+)-10-Camphorsulfonate (20ab).[12] Ester **19ab** (87 mg, 0.43 mmol) was treated with (+)-10-camphorsulfonyl chloride (125 mg, 0.499 mmol) in C_5H_5N (0.5 mL, room temperature, 12 h), evaporated, and separated (silica gel, $CHCl_3-EtOAc$ (9:1)) to give a mixture of epimers, **20ab** (131.8 mg, 31.5 mmol, 73%, colorless oil). Anal. Calcd for $C_{21}H_{36}O_6S$: C, 60.56; H, 8.71; S, 7.69. Found: C, 60.35; H, 8.82; S, 7.52.

Conversion of Natural Ester 19a to Its (+)-10-Camphorsulfonate (20a). The natural ester **19a** (890 μg, 4.7 μmol) was converted as above and purified by HPLC (Cyano column, hexane–2-propanol (20:1)) to give pure oily **20a** (950 μg, ca. 49%): ^1H NMR ($CDCl_3$, 300 MHz, Figure 2c).

Epimeric Carbamates 21ab.[13] A mixture of (R)-α-methylbenzyl isocyanate (668 mg, 4.5 mmol) and **19ab** (850 mg, 4.1 mmol) in CH_2Cl_2 (2 mL) and C_5H_5N (0.5 mL) was heated at reflux for 42 h. Purification (silica gel, hexane–2-propanol (20:1)) gave a mixture of epimers **21ab** (1.19 g, 78%): CIMS m/z (relative intensity) 350 (M +

(29) Witten, J. L.; Schaffer, M. H.; O'Shea, M.; Cook, J. C.; Hemling, M. E.; Rinehart, K. L., Jr. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 350–358.

H, 100), 334 (12), 318 (8), 272 (2), 246 (43), 233 (37), 203 (43), 185 (95), 164 (60), 153 (33), 105 (80), 85 (35), 71 (30), 59 (38). Anal. Calcd for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01. Found: C, 69.00; H, 9.03; N, 3.90.

Diastereomeric mixture **21ab** (70 mg) was separated by HPLC (phenyl column, hexane–2-propanol (60:1) to afford optically pure **21b** (28 mg) as the less polar isomer, an oil: $[\alpha]^{20}_D$ +33.8° (c 2.80, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.30 (5 H, m), 5.07 (1 H, m), 4.95 (1 H, br d, J = 6.0), 4.92 (1 H, br m), 3.68 (3 H, s). The more polar fraction gave the other optically pure carbamate **21a** (29 mg): $[\alpha]^{20}_D$ +36.6° (c 2.88, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.30 (5 H, m), 5.06 (1 H, m), 4.95 (1 H, br d, J = 6.0), 4.88 (1 H, br m), 3.56 (3H, s).

Cleavage of Carbamates 12a,b to Optically Pure Esters 19a,b.[14] To a solution of **21b** (22.5 mg, 64.4 μmol, benzene, 1 mL) were added Et_3N (20 μL, 143 μmol) and $SiHCl_3$ (25 μL, 138 μmol). The mixture stood at room temperature for 36 h, saturated aqueous NH_4Cl (1 mL) was added, and the organic layer was evaporated and purified (cyano column HPLC, C_6H_6–EtOAc (4:1)), giving optically pure **19b** (S-isomer) (4 mg, 33%) as a colorless oil: $[\alpha]^{20}_D$ +18.4° (c 0.243, $CHCl_3$). Isomer **21a** (24.2 mg) was cleaved similarly to give optically pure **19a** (R-isomer) (7.5 mg, 54%) as a colorless oil: $[\alpha]^{20}_D$ −18.4° (c 0.565, $CHCl_3$) (lit.[15] $[\alpha]^{22}_D$ −18.5°, $CHCl_3$).

Optically Pure Camphorsulfonyl Esters 20a,b. Optically pure **19a,b** were separately converted as above to give optically pure **20a** (3R-isomer, colorless oil): $[\alpha]^{24}_D$ +14.4° (c 1.20, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.12 (1 H, ddt, J = 6.1, 6.1, 6.1), 3.72 (3 H, s), 3.67 (1 H, d, J = 15.0), 3.00 (1 H, d J = 15.0) (Figure 2b). Anal. Calcd for $C_{21}H_{36}O_6S$ (M + H): M_r 417.2311. Found: M_r 417.2308 (HRFABMS).

Optically pure **20b** (3S-isomer, colorless oil): $[\alpha]^{24}_D$ −25.4° (c 1.36, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.12 (1 H, ddt, J = 6.1, 6.1, 6.1), 3.72 (3 H, s), 3.59 (1 H, d, J = 15.0), 3.09 (1 H, d, J = 15.0) (Figure 2a).

(R)-(3-Hydroxydecanoyl)-(Gln)$_4$-OH (22). A suspension of **4** (6.0 mg) was hydrolyzed under the same conditions as **3** to give a DMSO-soluble solid (**22**, 2.3 mg): FABMS m/z 701.2 (M + H), 683, 573. This compound was hydrolyzed without further purification.

Acid Hydrolysis of 22 Followed by Derivatization for HPLC Analyses. Compound **22** (1 mg) was hydrolyzed (6 N HCl, 0.5 mL, 12 h, 100 °C) and extracted (CH_2Cl_2). The organic layer was treated with acidic methanol (MeOH–AcCl (9:1), 100 °C, 30 min) to give an oil, **19a**. A portion of the oil (2/3, less than 50 μg) in C_6H_6 (50 μL) was treated with (R)-(+)-α-methylbenzyl isocyanate (500 μg) and Et_3N (50 μL) at 90 °C for 28 h in a sealed vial. Solvent was evaporated (N_2) to give an oil containing carbamate **21a**: CIMS m/z 350, 246, 203, 185. Anal. Calcd for $C_{20}H_{32}NO_4$ (M + H): M_r 350.2332. Found: M_r 350.2331 (HRCIMS). The oil was subjected to HPLC analysis on an analytical phenyl column using a UV detector (254 nm) and then on an analytical cyano column with hexane–2-propanol (50:1).

Treatment of 3 and 4 with I,I-[Bis(trifluoroacetyl)]iodobenzene (BTI).[16] Compound **3** (2 mg) was treated with BTI (10 mg) in CH_3CN–H_2O (1:1) at room temperature for 48 h and concentrated to an aqueous mixture, from which the excess reagent was extracted with C_6H_6. The aqueous layer was concentrated to give white amorphous **23**: mp 152–158 °C; $[\alpha]^{25}_D$ −65° (c 1.4, $CHCl_3$); IR (film) 3300, 3060, 2960, 2880, 1738, 1637, 1514, 1458, 1204, 1179 cm^{-1}; FABMS m/z 1583. Anal. Calcd for $C_{79}H_{132}N_{13}O_{20}$ (M + H): M_r 1582.9712. Found: M_r 1582.9702 (HRFABMS).

Similarly, compound **4** (2 mg) was treated to give white amorphous **24**: mp 158–162 °C; $[\alpha]^{25}_D$ −81° (c 0.6, MeOH); IR (film) 3300, 3060, 2960, 2874, 1740, 1635, 1514, 1450, 1240, 1169 cm^{-1}; FABMS m/z 1684. Anal. Calcd for $C_{83}H_{141}N_{15}O_{21}$: M_r 1684.0426 (M + H). Found: M_r 1684.0431 (HRFABMS).

Isolation of Didemnin M (1). A part of fraction A (21 g) was partitioned between the upper and lower layers of EtOAc–heptane–MeOH–H_2O (7:4:4:3) to give a green gum (12 g, lower layer) which was chromatographed (silica gel, 0.6 kg, $CHCl_3$–MeOH (8:1), 12 fractions). A portion (270 mg) of fraction 5 was further separated (silica gel, 60 g, with $CHCl_3$–MeOH (8:1)) into nine fractions (fractions 5-1 to 5-9). Fraction 6 (26 mg) was passed through a short silica gel column (70-230 mesh, 1 g, treated with ammonia gas prior to use) with $CHCl_3$–MeOH (4:1). The resulting solid was purified by HPLC (C-18 column, MeOH–H_2O (10:1)) to give **1** (10 mg, white powder): mp 158–160 °C; $[\alpha]^{25}_D$ −68.4° (c 1.1, $CHCl_3$); IR (film) 3340, 2960, 1734, 1637 cm^{-1}; UV (CH_3OH) λ_{max} 204 (log ϵ 4.76), 224 (4.38); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.95 (1 H, d, J = 8.7), 7.40 (1 H, J = 9.3), 7.28 (1 H, br s), 7.07 (2 H, d, J = 8.4), 6.84 (2 H, d, J = 8.4), 6.62 (1 H, br s), 5.81 (1 H, br s), 3.79 (3 H, s), 3.40 (3 H, s), 2.54 (3 H, s), 1.48 (3 H, d, J = 6.9), 1.40 (3 H, d, J = 6.3), 1.33 (3 H, d, J = 6.6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.7, 178.6, 176.3, 173.1, 172.4, 172.1, 172.0, 171.2, 171.1, 170.4, 169.6, 169.5, 168.3, 158.6, 130.3, 129.8, 114.1, 81.4, 71.0, 70.9, 70.5, 69.3, 67.5, 66.2, 59.0, 57.2, 56.8, 56.6, 56.5, 55.9, 55.3, 54.3, 53.4, 51.8, 49.7, 49.6, 47.0, 41.3, 38.6, 37.3, 36.2, 34.1, 33.9, 31.2, 31.0, 29.3, 28.8, 27.9, 26.9, 25.7, 25.6, 25.5, 25.3, 25.0, 24.8, 24.5, 23.8, 23.6, 21.3, 20.9, 18.7, 16.7, 15.9, 15.2, 14.8, 11.6; FABMS m/z 1352 (M + H), 536, 381, 312, 240. Anal. Calcd for $C_{67}H_{103}N_{10}O_{19}$: M_r 1351.7401 (M + H). Found: M_r 1351.7392 (HRFABMS). Compound **1** was hydrolyzed, derivatized, and analyzed by GC (conditions A).

Basic Hydrolysis of 1. To compound **1** (3.4 mg) in methanol (0.5 mL) was added Na_2CO_3 (1 N, 10 μL) at room temperature. The mixture was neutralized (1 N HCl) immediately after the addition of base, and $CHCl_3$ was added to the suspension. The organic layer was dried, concentrated, and separated by HPLC (MeOH–H_2O (7:1)) to give **9**.

Isolation of Didemnin N (2), Nordidemnin N (5), Epididemnin A$_1$ (6), and Acyclodidemnin A (7). Fraction A (97 g) was partitioned as above. The lower layer (50 g) was chromatographed (silica gel, 1.2 kg, $CHCl_3$–MeOH (8:1)) into 13 fractions (fractions 1–13). Fraction 4 (1.05 g) was further separated by HSCCC with EtOAc–cyclohexane–toluene–MeOH–H_2O (7:2:2:4:4) into five fractions (fractions 4-1 to 4-5), using the upper layer as the mobile phase with a flow rate of 2 mL/min. Fraction 4-2 (378 mg) was then separated on a Sephadex LH-20 column with MeOH into fractions C–G. Fraction E was further separated by a silica gel column (TLC grade gel) with EtOAc–2-propanol (10:1) followed by HPLC using a silica gel column with EtOAc–2-propanol (25:1) to give **2** (17.8 mg) as a first peak. The second peak was purified by reversed-phase C-18 HPLC with MeOH–H_2O (7:2) to give **5** (3.2 mg).

Didemnin N (2), a yellowish solid, showed the following physical properties: mp 150–152 °C; $[\alpha]^{24}_D$ −49° (c 1.6, $CHCl_3$); IR (film) 3333, 2959, 1734, 1635 cm^{-1}; UV (CH_3OH) λ_{max} 224 (log ϵ 3.98), 277 (3.08) nm; ^1H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) δ 205.0, 173.6, 173.0, 172.5, 172.4, 171.7, 170.5, 170.0, 169.6, 169.1, 155.4, 130.5, 127.9, 115.5, 81.1, 71.5, 67.3, 65.9, 60.6, 57.4, 56.9, 55.3, 54.9, 54.5, 49.3, 49.0, 47.2, 47.1, 41.5, 38.7, 36.2, 34.9, 33.4, 31.2, 31.1, 29.0, 28.3, 27.5, 25.9, 25.0, 24.8, 24.7, 23.4, 21.3, 20.8, 20.1, 20.0, 18.8, 16.7, 15.7, 15.2, 13.6, 11.8; FABMS see Table 2. Anal. Calcd for $C_{55}H_{86}N_7O_{15}$: M_r 1084.6182 (M + H). Found: M_r 1084.6187 (HRFABMS).

Nordidemnin N (5), white powder, showed the following physical properties: mp 154–156 °C; $[\alpha]^{24}_D$ −54° (c 0.13, $CHCl_3$); IR (film) 3323, 2959, 1734, 1635, 752 cm^{-1}; UV (CH_3OH) λ_{max} 224 sh (log ϵ 4.04), 277 (3.20) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 7.87 (1 H, d, J = 9.0), 7.58 (1 H, d, J = 7.0), 7.16 (1 H, br d, J = 10.0), 6.99 (2 H, J = 8.5), 6.74 (1 H, d, J = 8.5), 5.95 (1 H, d, J = 8.0), 5.40 (1 H, dd, J = 3.0, 11.5), 5.20 (1 H, d J = 3.0), 5.02 (1 H, m), 4.77 (3 H, m), 4.68 (1 H, br d, J = 5.0), 4.44 (1 H, q, J = 7.5), 4.40 (1 H, q, J = 7.0), 4.24 (2 H, m), 4.02 (1 H, t, J = 9.5), 3.92 (1 H, dt, J = 3.5, 9.5), 3.70 (2 H, m), 3.6 (1 H, m), 3.47 (1 H, m), 3.14 (3 H, s), 1.40 (3 H, d, J = 7.0), 1.39 (3 H, d, J = 7.0), 1.30 (3 H, d, 7.0); FABMS see Table 2. Anal. Calcd for $C_{54}H_{84}N_7O_{15}$: M_r 1070.5967 (M + H). Found: M_r 1070.5996 (HRFABMS).

Epididemnin A$_1$ (6). Fraction 12 (650 mg) was chromatographed (silica gel (70–230 mesh), 85 g, $CHCl_3$–MeOH (4:1 → 1:1), gradient) into eight fractions. Fraction 12-1 (145.5 mg) was separated on a silica gel column with EtOAc–2-propanol (15:1). Fraction 12-1-5 (9.2 mg) was purified on silica gel (2–10 μm, $CHCl_3$–MeOH (8:1)) to give **6** as a white powder (4.8 mg): mp 130–132 °C; $[\alpha]^{23}_D$ −100° (c 0.13, $CHCl_3$); IR (film) 3330, 2960, 2874, 1738, 1659, 1649, 1514, 1456, 1169 cm^{-1}; UV (CH_3OH) λ_{max} 206 (log ϵ 4.69) 229 (4.49) nm; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1. Anal. Calcd for $C_{49}H_{79}N_6O_{12}$: M_r 943.5756 (M + H). Found: M_r 943.5776 (M + H) (HRFABMS).

Acyclodidemnin A (7). Fraction 12-2 was purified by HPLC (C-18) with MeOH–H_2O (8:1) to give **7** (5.8 mg), a white powder: mp

126–130 °C: $[\alpha]^{26}_{D}$ −71° (c 0.06, $CHCl_3$); IR (film) 3300, 2960, 1732, 1635, 1514, 1456, 1263 cm^{-1}; UV (CH_3OH) λ_{max} 206 (log ϵ 4.80), 230 (4.51) nm; FABMS m/z 961 (M + H); FABMS/CID/MS m/z 961, 943, 834, 770, 752, 655, 627, 541, 528, 368, 307, 210, 100, 70. Anal. Calcd for $C_{49}H_{81}N_6O_{13}$: M_r 961.5862 (M + H). Found: M_r 961.5871 (M + H) (HRFABMS).

***N,O,O*-Triacetylacyclodidemnin A (7a).** Compound **7** (0.86 mg) was treated with acetic anhydride (0.1 mL) in C_5H_5N (0.1 mL) at room temperature for 12 h and evaporated. The resulting product, **7a**, was subjected to analysis by FABMS (m/z 1088) and FABMS/CID/MS (m/z 1088 → 1070, 919, 782, 321, 307, 210, 170, 142, 100, 70). Anal. Calcd for $C_{55}H_{87}N_6O_{16}$: M_r 1087.6179 (M + H). Found: M_r 1087.6201 (HRFABMS).

Mitsunobu Reaction of 9. Imides 25 and 26.[20] A solution of diethyl azodicarboxylate (16 mg, 0.092 mmol) in THF (0.5 mL) was added to a mixture of **9** (20 mg, 0.018 mmol), PPh_3 (23.6 mg, 0.090 mmol), phthalimide (13.2 mg, 0.090 mmol), and THF (0.5 mL) over 15 min. The reaction was monitored by TLC for 24 h at room temperature. The reaction mixture was concentrated (N_2), and the resulting solid was separated (silica gel column, EtOAc). The polar fraction gave imide **25** (13.5 mg, 60%), a pale yellow solid: mp 158–159 °C; $[\alpha]^{24}_{D}$ −31.9° (c 1.06, CH_3OH); IR (film) 3330, 2960, 1720, 1640 cm^{-1}; UV (CH_3OH) λ_{max} 214 nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.94 (1 H, d, J = 9.5), 7.83 (2 H, m), 7.77 (1 H, d, J = 6.0), 7.69 (2 H, m), 7.30 (1 H, d, J = 10.0), 7.04 (2 H, d, J = 8.5), 6.83 (2 H, d, J = 8.5), 3.78 (3 H, s), 3.20 (3 H, s), 2.54 (3 H, s). Anal. Calcd for $C_{65}H_{93}N_8O_{16}$ (M + H): M_r 1241.6734. Found: M_r 1241.6710 (HRFABMS). A portion of **25** (1 mg) was hydrolyzed, derivatized, and analyzed on GC (conditions A).

The less polar fraction gave imide **26** (4.3 mg, 20%), a pale yellow powder: mp 158 °C; IR (film) 3393, 2974, 2897, 1730, 1651, 1380, 1452, 1252 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.15 (1 H, d, J = 9.0, NH), 7.84 (2 H, m, Phth), 7.80 (1 H, d, J = 10.0, NH), 7.70 (2 H, m, Phth), 7.64 (1 H, d, J = 7.0, NH). 7.04 (2 H, d, J = 8.5, Me_2Tyr), 6.30 (1 H, dd, J = 15.5, 3, anhydro Ist), 6.83 (2 H, d, J = 8.5 Me_2-Tyr), 6.72 (1 H, dd, J = 15.5, 2.5, anhydro Ist), 3.78 (3 H, s), 3.17 (3 H, s), 2.56 (3 H, s). Anal. Calcd for $C_{65}H_{91}N_8O_{15}$: M_r 1223.6604 (M + H). Found: M_r 1223.6632 (HRFABMS).

Mitsunobu Reaction of 2. A sample of **2** (4.5 mg, 0.042 mmol) was treated with 10 equiv of reagents by following the procedure described above. The product was separated on a silica gel column to give a solid. Anal. Calcd for $C_{63}H_{87}N_8O_{15}$: M_r 1195.6291 (M + H). Found: M_r 1195.6306 (HRFABMS). A portion of the product (1 mg) was hydrolyzed, derivatized, and analyzed by GC (conditions A).

Ethyl (4*S*)-4-(Benzyloxy)-3-hydroxy-2,5-dimethylhexanoate (29a–32a). A solution of $NaBH_4$ (20 mg, 0.53 mmol) in THF–H_2O (1:1) 6 mL was added to a stirred solution of ethyl *O*-benzyl-α-(α-hydroxyisovaleryl)propionate (*O*-Bzl-Hip-OEt, 151.5 mg, 0.52 mmol)[22] in THF over 12 min at −4 °C. The reaction mixture was stirred at room temperature for 3 h, HCl (1 N, 0.52 mL) was added, and the product was extracted with CH_2Cl_2 (25 mL × 2). The organic layer was dried over sodium sulfate to give an oil (137 mg) upon evaporation of the solvents. The oil was chromatographed (silica gel gravity column with cyclohexane–EtOAc (4:1)) to give a mixture of diastereomeric alcohols **29a–32a** (107.5 mg, 69.9%). Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.45; H, 8.94.

A portion (84.6 mg) of the mixture was separated first by HPLC (C-18, MeOH–H_2O (7:1)) to give **31a** (2*S*,3*R*,4*S*) (3.64 mg) as the second peak, an oil: $[\alpha]^{24}_{D}$ +22° (c 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4–7.2 (5 H, m, Bzl), 4.59 (2 H, s, BzlCH_2), 4.0 (2 H, m, O–CH_2–CH_3), 3.63 (1 H, ddd, J = 10.0, 6.5, 3.0, H-3), 3.24 (1 H, dd, J = 6.0, 6.0, H-4), 2.83 (1 H, dq, J = 3.0, 7.5, H-2), 2.05 (1 H, sext, 4.8, 6.6), 1.32 (3 H, d, J = 7.5, CH_3-2), 1.19 (3 H, t, J = 7.2, O–CH_2–CH_3), 1.00 (6 H, d, J = 6.6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.2, 138.6, 128.4, 128.3, 127.7, 86.8, 74.9, 74.7, 60.5, 39.1, 29.9, 19.8, 17.6, 15.8, 14.0.

The first peak was a mixture of three other isomers. HPLC (silica gel, hexane–EtOAc (8:3)) of a portion of the mixture gave **30a**, **32a**, and **29a**.

30a (2*S*,3*S*,4*S*, 5.74 mg), an oil: $[\alpha]^{24}_{D}$ + 29° (c 0.57, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4–7.2 (5 H, m, Bzl), 4.62 (2 H, ABq, J = 10.8, BzlCH_2), 4.0 (2 H, m, O–CH_2–CH_3), 3.77 (1 H, ddd, J = 7.8, 7.8, 2.1, H-3), 3.09 (1 H, dd, J = 2.1, 6.3, H-4), 2.61 (1 H, dq, J = 7.2, 7.2, H-2), 2.03 (1 H, m), 1.25 (3 H, d, J = 6.3, CH_3-2), 1.24 (3 H, t, J = 7.2, O–CH_2–CH_3), 1.00 (3 H, d, J = 6.6), 0.99 (3 H, J = 6.6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.1, 138.2, 128.4, 127.7, 84.1, 74.4, 72.3, 60.4, 44.2, 30.2, 19.0, 18.4, 14.2, 13.7.

32a (2*R*,3*S*,4*S*, 35.0 mg), an oil: $[\alpha]^{24}_{D}$ −8.1° (c 3.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4–7.2 (5 H, m, Bzl), 4.65 (2 H, ABq, J = 9.0, BzlCH_2), 4.8 (2 H, q, J = 7.2, O–CH_2–CH_3), 3.76 (1 H, br t, J = 7.8, H-3), 3.19 (1 H, dd, J = 1.5, 6.6, H-4), 2.80 (1 H, d, J = 6.3, OH), 2.63 (1 H, dq, J = 6.9, 6.9, H-2), 2.11 (1 H, m), 1.25 (3 H, t, J = 6.3, O–CH_2–CH_3), 1.11 (3 H, d, J = 6.6, CH_3-2), 1.00 (6 H, d, J = 6.6); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 175.3, 138.1, 128.3, 127.5, 82.9, 73.6, 73.3, 60.4, 43.9, 29.7, 18.9, 18.6, 14.2, 14.0.

29a (2*R*,3*R*,4*S*, 9.68 mg), an oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4–7.2 (5 H, m, Bzl), 4.60 (2 H, ABq, J = 11.1, BzlCH_2), 4.10 (2 H, q, J = 7.2, O–CH_2–CH_3), 4.02 (1 H, m, H-3), 3.25 (1 H, dd, J = 3.0, 7.8, H-4), 2.82 (1 H, dq, J = 3.0, 6.6, H-2), 2.70 (1 H, d, J = 3.6, OH), 2.12 (1 H, m), 1.24 (3 H, t, J = 7.2, O–CH_2–CH_3), 1.22 (3 H, d, J = 7.5, CH_3-2), 1.06 (3 H, d, J = 6.6), 1.01 (3 H, d, J = 6.9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.3, 138.1, 128.3, 127.5, 82.9, 73.6, 73.3, 60.4, 43.9, 29.7, 18.9, 18.6, 14.2, 14.0.

2,3-Dihydro-4-isopropyl-2-methyltetronic Acid, γ-Lactones A (29–32). A mixture of alcohol **32a** (16.7 mg, 0.057 mmol) and Pd on activated carbon (10%, 47.5 mg) in MeOH (2 mL) was stirred in a H_2 atmosphere 30 min at room temperature, filtered through a Sep-Pak (C-8) column with MeOH, and concentrated to give an oil (11.0 mg). A portion of the oil (3.7 mg) was treated with a mixture of C_6H_6–TFA (100:1) in a sealed sample vial at 110 °C for 15 min to give lactone **32**:[23] ^1H NMR ($CDCl_3$, 300 MHz) δ 4.38 (1 H, dd, J = 2.7, 4.4), 3.82 (1 H, dd, J = 9.9, 2.7), 2.74 (1 H, dq, J = 4.4, 7.2), 2.11 (1 H, m), 1.26 (3 H, d, J = 7.2), 1.12 (3 H, d, J = 6.3), 0.97 (3 H, d, J = 6.6). Anal. Calcd for $C_8H_{15}O_3$: M_r 159.1018 (M + H). Found: M_r 159.1021 (M + H) (HRCIMS).

Other isomers were treated in the same manner to give the corresponding lactones **29–31**.

29 (needles):[23] ^1H NMR (C_6D_6, 500 MHz) δ 3.29 (1 H, t, J = 6.8), 3.12 (1 H, dd, J = 9.0, 6.8), 1.98 (1 H, dq, J = 9.0, 7.0), 1.48 (1 H, sext, J = 7.0), 1.00 (3 H, d, J = 7.2), 0.84 (3 H, d, J = 6.5), 0.77 (3 H, d, J = 6.5). Anal. Calcd for $C_8H_{15}O_3$: 159.1018 (M + H). Found: 159.1021 (HRCIMS).

30 (needles):[23] ^1H NMR ($CDCl_3$, 500 MHz) δ 4.16 (1 H, br t, J = 3.0), 4.03 (1 H, dd, J = 9.9, 3.0), 2.64 (1 H, q, J = 8.0), 2.16 (1 H, m), 1.29 (3 H, d, J = 8.0), 1.12 (3 H, d, J = 6.5), 0.98 (3 H, d, J = 6.5). Anal. Calcd for $C_8H_{15}O_3$: M_r 159.1018 (M + H). Found: M_r 159.1021 (HRCIMS).

31 (needles): ^1H NMR ($CDCl_3$, 300 MHz) δ 4.32 (1 H, dd, J = 1.5, 6.5), 4.04 (1 H, dd, J = 1.5, 8.1), 2.72 (1 H, quint, J = 7.0), 1.81 (1 H, sext, J = 7.0), 1.27 (3 H, d, J = 7.5), 1.00 (6 H, d × 2 overlap). Anal. Calcd for $C_8H_{15}O_3$: M_r 159.1018 (M + H). Found: M_r 159.1021 (HRCIMS).

Melting points, [α]'s, and ^{13}C NMR data for **29–32** are listed in Table 3. Lactones **29–32** were derivatized (TFA/TFAA, 90 °C, 20 min) and analyzed on GC (conditions B).

Dihydrodidemnin A (27). A solution of $NaBH_4$ (3.50 mg, 0.095 mmol) in THF–H_2O (1:1) (2 mL) was added dropwise to a solution of **8** (79.4 mg, 0.084 mmol) in THF (2 mL) at 0 °C. The mixture was stirred at 0 °C for 50 min, the temperature was raised to room temperature over 2 h, HCl (1N, 90 μL) was added to the solution, and the product was extracted with CH_2Cl_2. The organic layer was concentrated, then separated (silica gel, $CHCl_3$–MeOH (6:1)), to give pure **27** (53.4 mg, 67%). Anal. Calcd for $C_{49}H_{81}N_6O_{12}$: M_r 945.5912 (M + H). Found: M_r 945.5934 (HRFABMS).

Dihydrodidemnin B (37). An aqueous solution (100 μL) of $NaBH_4$ (1 mg/mL) was added to a solution of **9** (1 mg) in THF (0.5 mL). The mixture was stirred at room temperature for 12 h, HCl (1N, 9 μL) was added, and organic solvent was removed by N_2. The product was extracted with CH_2Cl_2 to give a solid, which was separated by HPLC (C-18) with MeOH–H_2O (7:1) to give **37** (260 μg, 47% conversion) and unreacted **9** (450 μg). Anal. Calcd for $C_{57}H_{92}N_7O_{15}$: M_r 1114.6651 (M + H). Found: M_r 1114.6657 (HRFABMS).

Dihydrodidemnin N (38). Compound **2** (960 μg) was reduced as above to give **38** (200 μg) and unreacted **2** (330 μg). Anal. Calcd for

$C_{55}H_{88}N_7O_{15}$: M_r 1086.6339 (M + H). Found: M_r 1086.6369 (HRFABMS).

Dihydroepididemnin A$_1$ (39). A solution of **6** (930 μg) was reduced as above to give a mixture of **39** and hydrated (presumably ring opened) compound: FABMS m/z 963 (M + H), 945 (M + H). The mixture was carried to the next step without further purification.

Compounds **27**, **37**, **38**, and **39** were hydrolyzed separately (6 N HCl, 80 °C, 12 h), derivatized, and analyzed by GC (conditions C).

Chiral TLC Analyses of Me$_2$Tyr and MeLeu in 6. Compound **6** (150 μg) was hydrolyzed (6 N HCl, 110 °C, 17 h). The hydrolyzate was concentrated by N_2 and chromatographed on C-18 HPLC (Analtech, 0.5 × 25 cm, 5 μm) with H_2O.[30] Each peak corresponding to Thr, Pro, Leu, MeLeu, and Me$_2$Tyr was collected (UV 205 nm, peaks identified by comparison with those of authentic samples). The separated samples of MeLeu and Me$_2$Tyr were analyzed by chiral TLC.[21]

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(30) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Tetrahedron* **1991**, *47*, 2169−2180.

Supplementary Material Available: IR and UV spectra of **2**, **3**, **4**, and **5**; IR spectra of **1**, **6**, and **7**; ^{1}H NMR spectra for **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **9**; COSY spectra for **2** and **6**; HMQC spectrum for **2**; ^{13}C NMR spectra for **1**, **2**, **3**, and **6**; FABMS spectra for **1**, **2**, **3**, **4**, and **5**; FABMS/CID/MS spectra for **1**, **2**, **3**, **4**, and **5**; Chiral GC traces of derivatized D,L-Tyr and D,L-Ala and derivatized hydrolyzates of **1**, **3**, **4**, **5**, **6**, **8**, **9**, **12**, **14**, and **25** (37 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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