Didemnimides A−D: Novel, Predator-Deterrent Alkaloids from the Caribbean Mangrove Ascidian *Didemnum conchyliatum*

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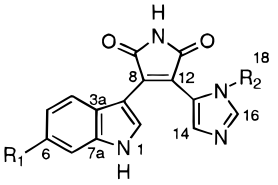
Four new alkaloids, didemnimides A−D (**1**−**4**), possessing a novel indole−maleimide−imidazole carbon skeleton, have been identified as the major predator deterrents found in the chemically-defended Caribbean mangrove ascidian *Didemnum conchyliatum*. The carbon skeleton of the didemnimides was identified by X-ray analysis of didemnimide A (**1**), while the structures of the related didemnimides B−D were subsequently assigned using combined spectral methods that emphasized one- and two-dimensional NMR methods. The didemnimides, in particular didemnimide D (**4**), are potent feeding deterrents against a natural assemblage of mangrove-specific carnivorous fish.

Marine ascidians, or sea squirts (Phylum Chordata, Class Ascidiacea), have proven to be a rich source of unique secondary metabolites that often possess potent and important bioactivities.[1,2] Colonial ascidians within the family Didemnidae have been particularly prolific, generally containing nitrogenous, amino acid-derived metabolites that have been extensively studied for their medical potential, particularly in the treatment of cancer.[3] Didemnid ascidians are known to elaborate metabolites representative of at least three major classes, cyclic peptides, pyridoacridine alkaloids, and β-carbolines. Recent examples of metabolites consistent with these structure classes are cyclodidemnamide, a weakly cytotoxic cyclic heptapeptide isolated by Toske *et al.* from *Didemnum molle*,[4] lissoclins A and B, polycyclic pyridoacridine alkaloids reported by Searle *et al.*, from *Didemnum* sp.,[5] and the didemnolides, N-substituted β-carbolines isolated by Schumacher *et al.* from an unidentified *Didemnum* species.[6] While much has been learned defining the potential drug applications of these molecules, very few studies have been performed to unravel their ecological roles in nature.[7]

As part of our ongoing biological and chemical studies of these animals, we encountered the tan, encrusting ascidian *Didemnum conchyliatum*, growing conspicuously in mangrove habitats on the blades of the Caribbean seagrass *Thalassia testudinum*.[8] Field and laboratory experiments performed using whole animals, and their organic extracts, indicated that the colonies were highly chemically defended against predation by carnivorous fish.[9] Using ecologically relevant bioassays[10] as a guide to fractionate the dichloromethane−methanol extract of the animal, four polar, orange and yellow compounds were readily detected in the active fractions. Final purification by replicate normal- and reversed-phase (C_{18}) column chromatography and HPLC methods led to the isolation of four new alkaloids, the didemnimides A−D (**1**−**4**, Chart 1), which defined the entire activity of the extract. The didemnimides are the first representatives of a novel class of combined indole−maleimide−imidazole alkaloids and, on structural grounds, can be hypothesized as derived via a condensation of tryptophan and histidine. The didemnimides differ in the presence of bromine on the indole ring and of methylation on the imidazole ring.

Chart 1



Didemnimide A (**1**): R_1=H, R_2=H

Didemnimide B (**2**): R_1=Br, R_2=H

Didemnimide C (**3**): R_1=H, R_2=CH_3

Didemnimide D (**4**): R_1=Br, R_2=CH_3

The structure of didemnimide A (**1**) was determined by a single-crystal X-ray experiment. Didemnimide A crystallized with difficulty from methanol/benzene mixtures as orange plates with one molecule of $C_{15}H_{10}N_4O_2 \cdot CH_3OH$ in the asymmetric unit. A computer-generated perspective drawing of the final X-ray model is shown in Figure 1. The molecule consists of planar indole, maleimide, and imidazole moieties that are

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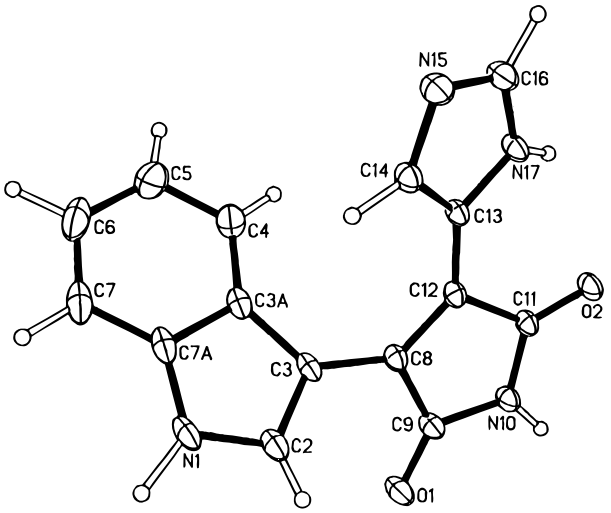

Figure 1. Computer-generated perspective drawing of the final X-ray model of didemnimide A (**1**). The methanol of crystallization is omitted for clarity.

twisted with respect to each other along the C_3-C_8 and $C_{12}-C_{13}$ σ bonds. In the crystalline state, the NH proton on the imidazole ring of didemnimide A (**1**) forms a strong H-bond with bridging methanol.[11]

The didemnimides A−D (**1**−**4**) were more easily recrystallized from acetonitrile and water mixtures. Their characteristic yellow-orange colors, and UV absorbances at approximately 420 nm, were consistent with an indole ring possessing additional conjugation at the C_3 position. The infrared spectra of the didemnimides A−D (**1**−**4**) in KBr showed the presence of amine protons (3000−3450 cm^{-1}) and showed the complex absorptions derived from the symmetric and asymmetric stretching of the imide carbonyl groups (1766−1755 cm^{-1}, ∼1700 cm^{-1} respectively).[12] In maleimides these frequencies are higher compared to acyclic imides due to ring strain and fall between 1792 and 1751 cm^{-1} (symmetric) and 1736 and 1686 cm^{-1} (asymmetric) with the asymmetric mode the most intense. Furthermore, the didemnimides showed a series of characteristic maleimide bands[13] at 1661−1610, 1550−1450, 1379−1333, 1080−1040, and 780−730 cm^{-1}. Comparison of high-resolution mass spectral data revealed that the didemnimides shared the indole−maleimide−imidazole carbon skeleton and only differed in the presence of bromine on the indole ring and of methylation on the imidazole ring. The dominant fragmentation pattern observed in the LREI mass spectra of the didemnimides was loss of the imide unit ($M^+ - m/z$ 71), competing with loss of the bromine when present. Following this, loss of the imidazole ring ($M^+ - m/z$ 67) competing with loss of the indole ring ($M^+ - m/z$ 115, didemnimide A, and $M^+ - m/z$ 116, didemnimide B) could be readily observed in the fragmentation patterns of didemnimides A and B. However, the LREIMS data for didemnimide B indicated that fragmentation to form a metastable ion (m/z 152) was preferred for this metabolite. Proton and carbon NMR data of the didemnimides, in particular didemnimide A, in dimethyl sulfoxide were complicated by chemical exchange and broadening and doubling of lines due to the tautomeric shift of hydrogen on the imidazole ring. The complicated spectra of didemnimide A (**1**) became somewhat simplified in pyridine, and all protons and carbons were subsequently confidently assigned using one- and two-dimensional NMR methods (Tables 1−4).

Didemnimide A (**1**) was obtained as irregular, orange needles (mp 234−235 °C). The ^1H NMR spectrum of these crystals in DMSO-d_6 showed all signals doubled and broadened, indicating the presence of two forms of didemnimide A (**1**). Given that the pK_a of the conjugate acid of the imidazole ring is approximately 7, both the protonated and nonprotonated forms of didemnimide A (**1**) appeared to be present. In pyridine-d_5, the equilibrium shifted to the free base; thus, several one- and two-dimensional NMR experiments, which were essential to confirm the NMR assignments of **1**, were recorded in pyridine-d_5. Table 1 shows the spectral data for the major species, the free base, of didemnimide A (**1**) in both solvents. In pyridine-d_5, the typical substitution pattern for a 3-substituted indole ring was readily observed (δ 7.64, d, 7.8 Hz; δ 7.18, dd, 7.8, 7.8 Hz; δ 7.27, dd, 7.8, 7.8 Hz; δ 7.55, d, 7.8 Hz; δ 8.48, s). The two remaining singlets (δ 8.18; δ 8.12) were assigned to the imidazole ring protons, whereas the NH protons of the imidazole and maleimide rings (p$K_a \approx 7.4$) were not observed in this solvent, presumably due to exchange. The connectivities of the indole and imidazole rings were fully established by long-range heterocorrelation NMR methods (HMBC experiment, performed at $J = 12$ Hz, see Table 1). The high-resolution EI mass spectrum of didemnimide A (**1**) indicated the molecular formula $C_{15}H_{10}N_4O_2$ (m/z 278.0793, $\Delta = 3.9$), and the UV absorbance at 430 nm ($\epsilon = 3000$) confirmed the extended conjugation through the maleimide ring. These spectral data were in full support of the structure assigned earlier by X-ray analysis.

Didemnimide B (**2**) was obtained as extremely fine, light orange needles (mp > 300 °C, dec). The structure of this metabolite was assigned by NMR using primarily HMQC and HMBC experiments (DMSO-d_6, see Table 2) and, due to the low number of multiple bond heterocorrelations observed, by comparison with didemnimide A (**1**), C (**3**), and D (**4**). High-resolution EI mass spectrometry measurements of didemnimide B (**2**) provided the molecular formula $C_{15}H_9N_4O_2{}^{79}Br$ (HREIMS m/z = 355.9904, $\Delta = 1.4$ ppm), indicating the presence of an aromatic bromine substituent in this molecule. Analysis of ^1H NMR data revealed the presence of a substituted indole ring (see Table 2), while the UV absorbance observed [420 nm ($\epsilon = 4300$)] was typical of the extended chromophore of this class of alkaloids. The position of bromine at C-6 on the indole ring was fully established by comprehensive analysis of the HMBC data for didemnimide B (**2**).

Didemnimide C (**3**) was obtained as dark orange needles (mp > 300 °C, dec), which illustrated a parent ion in the HREI mass spectrum at m/z = 292.0947, appropriate for the molecular formula $C_{16}H_{12}N_4O_2$ (Δ = 4.5 ppm). This formula indicated that didemnimide C (**3**) differed from didemnimide A (**1**) only in the substitution of a methyl group. Proton NMR data showed that the indole ring was unsubstituted (δ 6.38, d, 8.0 Hz; δ 6.77, dd, 8.0, 8.0 Hz; δ 7.10, dd, 8.0, 8.0 Hz; δ 7.44, d, 8.0 Hz; δ 8.07, s), while typical UV absorbance at 420 nm (ϵ = 5700) showed that the extended conjugation through

(11) Additional crystallographic parameters are available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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Table 1. NMR Assignments for Didemnimide A (1)

C no.	^{1}H (DMSO-d_6) δ (mult, J, no. of H's)[a,c]	^{1}H (pyr-d_5) δ (mult, J, no. of H's)[b,c]	^{13}C (DMSO-d_6) δ (no. of H's)[a]	^{13}C (pyr-d_5) δ (no. of H's)[a]	HMBC (pyr-d_5)[b,f]
1	12.45 (br s, 1H)	12.90 (br s, 1H)			138.0, 126.5, 106.3, 130.7
2	8.05 (br s, 1H)	8.48 (br s, 1H)	130.7 (CH)	130.7 (CH)	138.0, 126.5, 106.3
3			104.7 (C)	106.3 (C)	
3a			125.6 (C)	126.5 (C)	
4	7.07 (br m, 1H)	7.64 (br d, 7.8 Hz, 1H)	121.7 (CH)	123.0 (CH)	123.0, 138.0, 106.3
5	6.87 (br m, 1H)	7.18 (dd, 7.8, 7.8 Hz, 1H)	119.6 (CH)	120.8 (CH)	112.2, 126.5
6	7.07 (br m, 1H)	7.27 (dd, 7.8, 7.8 Hz, 1H)	121.7 (CH)	122.5 (CH)	123.0, 138.0
7	7.39 (br d, 7.8 Hz, 1H)	7.55 (d, 7.8 Hz, 1H)	112.0 (CH)	112.2 (CH)	128.6, 126.5
7a			136.5 (C)	138.0 (C)	
8			n.o.	n.o.	
9			172.9 (C)[d]	n.o.	
10	11.66 (br s, 1H)	n.o.			
11			172.8 (C)[d]	n. o.	
12			n.o.	n. o.	
13			125.7 (C)	127.2 (C)	
14	7.71 (br s, 1H)	8.18 (br s, 1H)	119.6 (CH)	128.6 (CH)	126.5, 138.0
15					
16	7.68 (br s, 1H)	8.12 (br s, 1H)	136.5 (CH)	138.0 (CH)	126.5, 128.6
17	10.87 (br s, 1H)	12.5 (br s, 1H)			

[a] ^{1}H NMR and ^{13}C NMR shifts were referenced to DMSO (^{1}H δ 2.49 and ^{13}C δ 39.5 ppm). Coupling constants are reported in Hz. [b] ^{1}H NMR and ^{13}C NMR shifts were referenced to pyridine (^{1}H δ 7.58 and ^{13}C δ 135.9 ppm). [c] Values of major species only. [d] Values may be interchanged. [e] Chemical shifts determined from the HMBC experiment; n.o. indicates no heterocorrelations observed. [f] HMBC data were optimized for $^nJ_{CH} = 12$ Hz.

Table 2. NMR Assignments for Didemnimide B (2)

C no.	^{1}H (DMSO-d_6) δ (mult, J, no. of H's)[a]	^{13}C (DMSO-d_6) δ (no. of H's)[a]	HMBC (DMSO-d_6)[b]
1	12.43 (br s, 1H)		
2	8.04 (s, 1H)	130.9 (CH)	
3		104.9 (C)	
3a		124.8 (C)	
4	7.02 (s, 1H)	121.6 (CH)	124.8, 136.7, 113.7
5	7.02 (s, 1H)	123.3 (CH)	123.3, 124.8, 113.7
6		113.7 (C)	
7	7.59 (s, 1H)	113.7 (CH)	113.7, 121.6
7a		136.7 (C)	
8		126.3 (C)[c]	
9		172.3 (C)[d]	
10	11.78 (br s, 1H)		
11		171.4 (C)[c]	
12		126.9 (C)[d]	
13		130.4 (C)	
14	7.76 (s, 1H)	119.9 (CH)	
15			
16	7.68 (s, 1H)	135.9 (CH)	130.4, 119.9
17	10.92 (br s, 1H)		

[a] ^{1}H and ^{13}C NMR shifts are referenced to DMSO (^{1}H δ 2.49 and ^{13}C δ 39.5 ppm). Coupling constants are reported in Hz. [b] HMBC data were optimized for $^nJ_{CH} = 8$ Hz. [c,d] Values may be interchanged.

Table 3. NMR Assignments for Didemnimide C (3)

C no.	^{1}H (DMSO-d_6) δ (mult, J, no. of H's)[a]	^{13}C (DMSO-d_6) δ (no. of H's)[a]	HMBC (DMSO-d_6)[b]
1	12.02 (br s, 1H)		131.8, 105.2, 125.0, 136.6
2	8.07 (s, 1H)	131.8 (CH)	105.2, 125.0, 136.6
3		105.2 (C)	
3a		125.0 (C)	
4	6.38 (d, 8.0 Hz, 1H)	119.9 (CH)	122.6, 135.6, 105.2
5	6.77 (dd, 8.0, 8.0 Hz, 1H)	120.8 (CH)	125.0, 112.45
6	7.10 (dd, 8.0, 8.0 Hz, 1H)	122.6 (CH)	119.9, 136.6
7	7.44 (d, 8.0 Hz)	112.5 (CH)	120.8, 125.0
7a		136.6 (C)	
8		134.1 (C)	
9		172.0 (C)[c]	
10	11.13 (br s, 1H)		172.0, 134.1, 171.8
11		171.8 (C)[c]	
12		134.1 (C)	
13		122.6 (C)	
14	7.07 (s, 1H)	131.8 (CH)	
15			
16	7.70 (s, 1H)	140.4 (CH)	
17			
18	3.15 (s, 3H)	32.3 (CH_3)	122.6, 140.4

[a] ^{1}H and ^{13}C NMR shifts are referenced to DMSO (^{1}H δ 2.49 and ^{13}C δ 39.5 ppm). Coupling constants are reported in Hz. [b] HMBC data were optimized for $^nJ_{CH} = 8$ Hz. [c,d] Values may be interchanged.

the maleimide ring was again intact. These overall data suggested that methylation had occurred on the imidazole ring at two possible locations, N-17 or N-15. The X-ray structure of didemnimide A (**1**) indicated that the position of the NH proton in the imidazole ring was at N-17 in the crystalline state. But, since imidazoles can be present in two tautomeric forms in solution, methylation could have occurred at either of the nitrogens. Methylation at N-17 was confirmed in didemnimide C (**3**) by long-range heterocorrelation NMR experiments. Strong HMBC correlations from the *N*-methyl protons (δ 3.15, s, H18) to a quaternary carbon (δ 122.6 (C)) assigned as C-13, and to a methine carbon (δ 140.4 (CH)), assigned as C-16, were apparent, suggesting *N*-methylation at N-17. Subsequently, the connectivities of the indole and maleimide rings were fully established by analogous HMBC methods (see Table 3).

Didemnimide D (**4**) was obtained as small, dark orange needles (mp > 250 °C, dec). High-resolution EI mass spectral analysis showed that **4** possessed the molecular formula $C_{16}H_{11}N_4O_2{}^{79}Br$ (HREIMS: m/z 370.0078, $\Delta = -3.4$ ppm), indicating that both bromination and *N*-methylation had occurred in this metabolite. An intense UV absorbance at 416 nm ($\epsilon = 4500$) confirmed that the extended conjugation of indole to maleimide to imidazole was also intact. The characteristic ^{1}H NMR pattern found in didemnimide D (**4**), an 8.7 Hz coupling between H-4 (δ 6.32, d) and H-5 (δ 6.92, dd), as well as a 1.8 Hz *meta* coupling between H-5 and H-7 (δ 7.64, d), suggested bromination at C-6 of the indole, as observed in **2**. This assignment was confirmed by HMBC experiments that confidently yielded all NMR assignments for this portion of the molecule (see Table 4). Likewise, the position of *N*-methylation was confirmed to be at N-17 on the basis of HMBC multiple bond heterocorrelations from the *N*-methyl protons (δ 3.15, s, H18) to the quaternary carbon (δ 122.8 (C)) assigned as C-13. The presence of imidazole and maleimide rings were also supported by comparison of NMR data with those derived from di-

Table 4. NMR Assignments for Didemnimide D (4)

C no.	^{1}H (DMSO-d_6) δ (mult, J, no. of H's)[a]	^{13}C (DMSO-d_6) δ (no. of H's)[a]	HMBC (DMSO-d_6)[b]
1	12.09 (br s, 1H)		
2	8.05 (br s, 1H)	132.5 (CH)	133.5, 105.3, 124.0, 137.6
3		105.3 (C)	
3a		124.0 (C)	
4	6.32 (d, 8.7 Hz, 1H)	121.6 (CH)	105.3, 137.6, 123.5
5	6.92 (dd, 8.7, 1.8 Hz, 1H)	123.5 (CH)	124.0, 115.3, 115.1
6		115.3 (C)	
7	7.64 (d, 1.8 Hz, 1H)	115.1 (CH)	123.5, 115.3, 137.6, 124.0
7a		137.6 (C)	
8		133.5 (C)	
9		172.0 (C)[c]	
10	11.18 (br s, 1H)		
11		171.8 (C)[c]	
12		120.0 (C)	
13		122.8 (C)	
14	7.07 (br s, 1H)	132.5 (CH)	
15			
16	7.72 (br s, 1H)	140.4 (CH)	
17			
18	3.15 (s, 3H)	32.2 (CH_3)	122.8

[a] ^{1}H and ^{13}C NMR shifts are referenced to DMSO (^{1}H δ 2.49 and ^{13}C δ 39.5 ppm). Coupling constants are reported in Hz. [b] HMBC data were optimized for $^nJ_{CH} = 8$ Hz. [c,d] Values may be interchanged.

demnimide C and by an HMBC correlation connecting the indole ring at C-2 to the maleimide ring at C-8. Due to the low number of multiple bond heterocorrelations observed, a quaternary carbon (δ 120.0 (C)) that did not show correlations in the HMBC experiment was assigned as C-12 by excluding all other possibilities.

The didemnimides are the first examples of a new alkaloid structural class and add to a relatively small group of naturally occuring maleimides. This group includes several microbial metabolites such as pencolide,[14] showdomycin,[15] and maleimycin,[16] isolated from *Penicillium* and *Streptomyces* species, and some plant metabolites like the hyperectines[17] from *Hypecoum leptocarpum*. An extensive class of staurosporine[18]-related protein kinase C inhibitors has been synthesized[19] for which the lead structures arcyriarubin A, a bis-indole maleimide, and arcyriaflavin A, an indole carbazole maleimide, were isolated from the fruiting bodies of the slime mold *Arcyria denudata*.[20] Arcyriaflavin A[21] and the polycitrins A and B,[22] *N*-substituted bisarylmaleimides, were also reported from the ascidians *Eudistoma* sp. and *Polycitor* sp., respectively. The didemnimides are most closely related to arcyriarubin A, and it is reasonable to assume that both classes of maleimides and the polycitrins are derived via similar biogenetic pathways. As proposed by Fröde *et al.*[23] and Terpin *et al.*,[24] this pathway appears to involve the coupling of two modified amino acid residues to form the central maleimide ring.

Whereas ascidian secondary metabolites have been extensively studied for their medical potential, their ecological functions have been less frequently investigated.[7] Ecologically relevant field and laboratory experiments[10] have now been designed to test the hypothesis that secondary metabolites function as predator deterrents. In this study, it was found that the didemnimides constitute a significant chemical defense against carnivorous fish in mangrove habitats. The most active didemnimide isomer, didemnimide D (**4**), deters feeding of the carnivorous wrasse *Thalassoma bifasciatum* at natural concentrations in aquarium assays. When tested in the mangrove environment itself, using an established method with control and treated foods, didemnimide D alone significantly reduced consumption relative to controls. Although didemnimides A−C showed somewhat lowered activity, the fact that their effects are additive in the extract suggests that the ascidian benefits from production of a mixture of didemnimide isomers with much greater potency overall.

Experimental Section

General Methods. NMR spectra were recorded at 500 MHz for ^{1}H and at 50 or 100 MHz for ^{13}C. ^{1}H NMR and ^{13}C NMR were referenced to solvent signals at 2.49 and 39.5 ppm for DMSO-d_6, or at 7.58 and 135.9 ppm for pyridine-d_5, respectively. Reversed-phase (C_{18}) HPLC was carried out on a semipreparative column (i.d. 10 mm) or on a preparative column (i.d. 20 mm) by monitoring UV single wavelength (254 nm). High- and low-resolution mass measurements were provided by the Mass Spectrometry Facility at the University of California, Riverside.

Isolation and Purification. *D. conchyliatum* was collected in 1990 and 1994 at a depth of 5−6 f from the blades of the seagrass *T. testudinum* in the mangrove channels of Sweetings Cay, near Grand Bahama Island, Bahamas. The freshly collected ascidians (~2100 mL) were separated from the grass blades and immediately extracted with 3 × 1.5 L portions of methanol/dichloromethane (1:1). The extracts were combined and reduced under vacuum to yield an aqueous methanol phase (~1040 mL), which was sequentially partitioned between isooctane, ethyl acetate, dichloromethane, and 2-propanol. The isooctane, dichloromethane, and ethyl acetate phases were combined, reduced to dryness, and fractionated by silica gel vacuum flash chromatography (Merck Type 60) employing a gradient of 0−5% methanol in dichloromethane. Five major fractions were obtained, one containing **1** and **2** and a less polar fraction containing **3** and **4**. The fraction containing **1** and **2** was further separated by reversed-phase (C_{18}) column chromatography (Crosfield Sorbsil C60 RP18B) using 35% water in methanol, followed by silica column chromatography (Merck Type 60) using 5% methanol in dichloromethane, and lastly reversed-phase (C18) HPLC (Rainin Dynamax C18, 60 Å, i.d. 10 or 20 mm) using acetonitrile and water mixtures. After collection from HPLC, **1** subsequently crystallized from the eluent (14% acetonitrile/water, yield 20 mg). Compound **2** was purified by reversed-phase (C_{18}) HPLC with 16% acetonitrile/water (yield 5 mg).

The fraction containing **3** and **4** was further separated by reversed-phase column chromatography (Crosfield Sorbsil C60

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RP18B) using 30% water in methanol, followed by silica column chromatography (Merck Type 60) using 5% methanol in dichloromethane, and lastly reversed-phase (C18) HPLC using acetonitrile and water mixtures. Compound **3** crystallized from the eluent (32% acetonitrile/water, yield 6 mg). Compound **4** eluted with 30% acetonitrile/water and was recrystallized from 25% acetonitrile/water (yield 7 mg).

Didemnimide A (1). X-ray Crystal Structure. Compound **1** crystallized with some difficulty from methanol and benzene as orange plates in space group $P\bar{1}$ with $a = 7.123(4)$ Å, $b = 10.303(4)$ Å, $c = 11.024(4)$ Å, $\alpha = 70.46(3)°$, $\beta = 84.36(4)°$, $\gamma = 72.20(3)°$ and one molecule of $C_{15}H_{10}N_4O_2 \cdot CH_3OH$ in the asymmetric unit. Crystallization was performed by allowing a solution of **1** in methanol to equilibrate with benzene in a closed chamber. A hemisphere of data was collected at −23 °C using θ:2θ scans and Cu Kα radiation. Of the 1992 symmetry-independent reflections surveyed, 1764 (80.9%, 4.0σ) were judged, observed, and used in refinement. All hydrogen atoms were added from difference maps, and the final R factor is 4.8%. The molecule consists of planar indole, maleimide, and imidazole moieties that are twisted with respect to each other (torsional angles $C_{12}-C_8-C_3-C_2$ of −141° and $C_8-C_{12}-C_{13}-N_{17}$ of −169°). In the crystalline state, the NH proton on the imidazole ring of didemnimide A (**1**) forms a strong H-bond with bridging methanol ($N_{17}H-O_3$, 1.931 Å; $N_{17}-O_3$, 2.788 Å).

Didemnimide A (1) was obtained as irregular orange needles (from acetonitrile/water) that showed the following physical and spectral properties: mp = 234−235 °C; UV λ_{max} (MeCN) 430 nm ($\epsilon = 3000$); IR (KBr) ν_{max} 3260, 3060, 2931, 2731, 1755, 1702, 1649, 1549, 1349, 1249, 1091, 750, 662, 620, 485 cm^{-1}; ^{1}H NMR (DMSO-d_6, Pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 50 MHz) see Table 1; LREIMS, m/z (rel intensity) 279 (14.8), 278 (91.4), 277 (100.0), 276 (10.0), 251 (17.1), 207 (14.4), 206 (11.2), 180 (9.0), 179 (15.4), 178 (4.3), 167 (7.0), 153 (23.3), 152 (14.1), 140 (7.0), 139 (6.1), 126 (13.0), 125 (11.3), 104 (9.4), 91 (21.2), 77 (11.8), 75 (10.6), 63 (6.8); HREIMS M$^+$ m/z = 278.0793 ($C_{15}H_{10}N_4O_2$, $\Delta = 3.9$ ppm).

Didemnimide B (2) was obtained as extremely fine, light orange needles (from acetonitrile/water) that showed the following physical and spectral properties: mp >300 °C dec; UV λ_{max} (MeCN) 420 nm ($\epsilon = 4300$); IR (KBr) ν_{max} 3248, 2943, 2719, 2367, 1755, 1702, 1655, 1543, 1343, 1249, 1091, 803, 656, 615, 591, 491 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 2; LREIMS m/z (rel intensity) 359 (14.5), 358 (91.4), 357 (45.7), 356 (90.6), 355 (34.0), 331 (13.7), 329 (18.8), 287 (20.9), 285 (20.0), 278 (22.3), 277 (100.0), 276 (79.6), 260 (10.2), 259 (13.7), 258 (11.6), 250 (12.9), 233 (24.9), 232 (13.3), 231 (30.4), 223 (13.1), 207 (12.2), 206 (32.2), 205 (21.6), 204 (13.3), 179 (32.3), 178 (25.7), 166 (13.1), 153 (13.8), 152 (65.1), 151 (42.8), 139 (16.4), 126 (10.6), 125 (27.4), 124 (12.8), 103 (10.6), 91 (11.3), 77 (13.7), 55 (16.3); HREIMS M$^+$ m/z = 355.9904 ($C_{15}H_9N_4O_2{}^{79}Br$, $\Delta = 1.4$ ppm).

Didemnimide C (3) was obtained as dark orange needles (from acetonitrile/water) that showed the following physical and spectral properties: mp >300 °C dec; UV λ_{max} (MeCN) 420 nm ($\epsilon = 5700$); IR (KBr) ν_{max} 3166, 3039, 2346, 1766, 1700, 1630, 1536, 1446, 1346, 1226, 1221, 1117, 1014, 913, 748, 667, 626, 500 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table 3; ^{13}C NMR (DMSO-d_6, 50 MHz) see Table 3; LREIMS m/z (rel intensity) 293 (17.2), 292 (100.0), 291 (47.5), 276 (7.9), 275 (5.2), 251 (26.4), 248 (12.6), 221 (29.3), 220 (27.7), 206 (5.3), 194 (9.8), 193 (16.0), 192 (6.0), 179 (17.3), 167 (14.0), 166 (20.3), 165 (10.5), 153 (21.2), 152 (22.5), 140 (10.7), 139 (15.2), 126 (10.1), 125 (12.9), 111 (6.7), 91 (7.4), 77 (5.7), 75 (6.1); HREIMS M$^+$ m/z = 292.0947 ($C_{16}H_{12}N_4O_2$, $\Delta = 4.5$ ppm).

Didemnimide D (4) was obtained as small dark orange needles (from acetonitrile/water) that showed the following physical and spectral properties: mp >250 °C dec; UV λ_{max} (MeCN) 416 nm ($\epsilon = 4500$); IR (KBr) ν_{max} 3448, 3225, 2966, 2367, 1766, 1702, 1637, 1543, 1449, 1384, 1343, 1261, 1232, 1114, 1085, 1055, 1008, 914, 808, 756, 662, 503 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table 4; ^{13}C NMR (DMSO-d_6, 50 MHz) see Table 4; EIMS m/z (rel intensity) 373 (21.0), 372 (94.5), 371 (41.9), 370 (100.0), 369 (20.1), 331 (19.3), 329 (27.1), 301 (15.1), 300 (10.5), 299 (16.1), 292 (17.1), 291 (35.8), 290 (40.8), 250 (10.5), 220 (18.1), 219 (13.8), 192 (13.1), 166 (14.5), 165 (15.4), 164 (11.2), 152 (16.0), 151 (16.3), 146 (10.4), 139 (14.1), 138 (10.5), 110 (11.2), 91 (11.2), 83 (13.9), 69 (14.1), 67 (10.0), 57 (22.1), 55 (28.6), 45 (15.4), 44 (34.8), 43 (42.0); HREIMS M$^+$ m/z = 370.0078 ($C_{16}H_{11}N_4O_2{}^{79}Br$, $\Delta = -3.4$ ppm).

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Supporting Information Available: ^{1}H and ^{13}C NMR, HMQC, HMBC, MS, UV, and IR data of **1**, **2**, **3**, and **4** (51 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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