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NOTE

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Didemnidines A and B, Indole Spermidine Alkaloids from the New Zealand Ascidian *Didemnum* sp.

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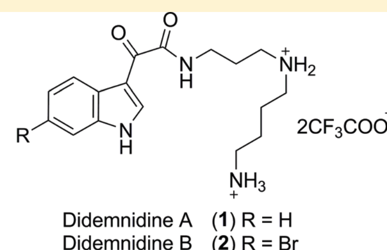
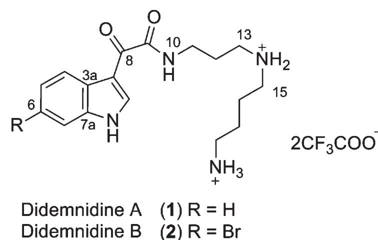
Supporting Information

ABSTRACT: Two new indole spermidine alkaloids, didemnidines A (**1**) and B (**2**), have been isolated from the New Zealand ascidian *Didemnum* sp. The structures of the metabolites, determined by analysis of 2D NMR spectra and confirmed via synthesis, embody an indole-3-glyoxylamide moiety linked to the N^1 position of spermidine, the latter motif being particularly rare among marine natural products. Didemnidine B and a synthetic precursor exhibited mild in vitro growth inhibition of *Plasmodium falciparum* with IC_{50}'s of 15 and 8.4 μM, respectively.



Marine natural product chemistry of ascidians of the family Didemnidae is dominated by amino acid-derived secondary metabolites,[1,2] which include cyclic peptides, pyridoacridine, β-carboline, lamellarin, 3,4-substituted maleimide, and polysulfide alkaloids. In many cases, these natural products exhibit biological activities with either human therapeutic or ecological relevance.[2] In the context of our search for potential anti-inflammatory lead molecules from New Zealand marine organisms,[3] we have investigated a mildly bioactive extract prepared from a *Didemnum* sp. ascidian collected at Tiwai Point, Southland, New Zealand. In addition to the known maleimides didemnimides A−D,[4] two new indole-3-glyoxylamidospermidine analogues, didemnidines A (**1**) and B (**2**), were isolated. The structures of both natural products were confirmed by synthesis, and they and two synthetic intermediates were tested for bioactivity in a range of anti-inflammatory, antitumor, and neglected disease whole parasite assays.



Extraction of the freeze-dried ascidian sample with MeOH, followed by fractionation utilizing C_8, C_{18}, and Sephadex LH-20 column chromatography, led to the purification of didemnimides A−D[4] and didemnidines A (**1**) and B (**2**). Our initial attempts to dereplicate the didemnimides via HPLC-DAD, ESIMS, and NMR were hampered somewhat by the fact that we had isolated the alkaloids as their TFA salts, whereas the original isolation report[4] presented data for the alkaloids as free bases. Considerable differences were observed for the ^{1}H NMR resonances assigned to imidazole proton H-16 ($\Delta\delta_{salt-free\ base}$ +1.0 to +1.4) and benzenoid proton H-4 ($\Delta\delta_{salt-free\ base}$ −0.3 to −0.4) between the salt and free base forms. Rigorous 2D-NMR spectroscopic assignments however determined the identities of the alkaloids (see Supporting Information for details).

Didemnidine A (**1**), isolated as a brown oil, displayed a pseudomolecular ion at m/z 317.1980 $[M + H]^+$ in the (+)-HRESI mass spectrum, establishing a molecular formula of $C_{17}H_{24}N_4O_2$. An absorption maximum at 328 nm in the UV spectrum suggested the presence of an indole moiety. The ^{1}H NMR spectrum of **1** (Table 1) exhibited resonances attributable to seven exchangeable protons (δ_H 12.29, 8.90, 8.54 (2H), 7.81 (3H)), five aromatic methines, and 14 alkyl protons. The ^{13}C NMR spectrum contained signals for all 17 carbons required by the molecular formula. Analysis of COSY NMR correlations established the presence of three separate proton spin systems: four protons (δ_H 8.23, 7.55, 7.27, and 7.25) were part of a 1,2-disubstituted benzene ring, two coupled proton resonances (δ_H

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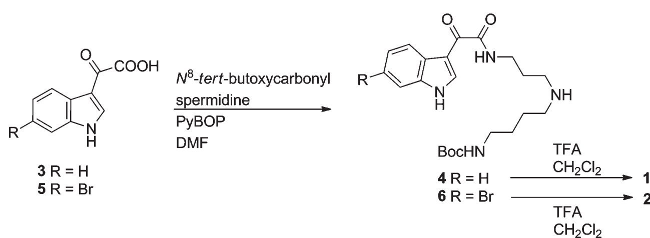
Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz), and HMBC NMR Data for Didemnidines A (**1**) and B (**2**)[a]

	didemnidine A (**1**)			didemnidine B (**2**)		
position	δ_C	δ_H (J in Hz)	HMBC[b]	δ_C	δ_H (J in Hz)	HMBC[b]
1		12.29, br s			12.39, s	
2	138.4	8.77, d (3.1)	3, 3a, 7a, 8	139.2	8.78, s	3, 3a, 7a
3	112.0			112.0		
3a	126.1			125.2		
4	121.1	8.23, m	3, 3a, 7a	122.8	8.15, d (8.4)	3, 3a, 6, 7a
5	122.5	7.25, m	3a, 7	125.4	7.41, dd (8.4, 1.5)	3a, 7
6	123.4	7.27, m	7, 7a	115.9		
7	112.5	7.55, m	3a, 7a	115.3	7.75, d (1.5)	5, 6, 7a
7a	136.2			137.1		
8	181.6			181.7		
9	163.7			163.3		
10		8.90, t (6.2)	9, 11		8.92, t (6.2)	9, 11
11	35.7	3.30 (obscured)[c]	12, 13	35.8	3.30 (obscured)[c]	12, 13
12	25.6	1.86, m	11, 13	25.6	1.85, m	11, 13
13	44.6	2.94, br s		44.6	2.94, br m	12
14		8.54, 2H, br s			8.49, 2H, br s	
15	46.0	2.94, br s		46.0	2.94, br m	16, 17, 18
16	22.5	1.60, br m	15, 17, 18	22.5	1.59, br m	15, 17
17	24.1	1.56, br m	15, 16, 18	24.1	1.59, br m	15, 16, 18
18	38.1	2.80, m	16, 17	38.1	2.81, br s	
19		7.81, 3H, br s			7.79, 3H, br s	

[a] Spectra recorded in DMSO-d_6 at 27 °C. [b] HMBC correlations, optimized for 8.3 Hz, are reported from the proton resonance to the indicated carbon resonance(s). [c] Resonance obscured by H_2O peak. The corresponding resonances for synthetic **1** and **2** were observed at δ_H 3.30 (dt, J = 6.2, 6.2 Hz, H_2-11). See Supporting Information.

12.29, 8.77) indicated the presence of a pyrrole ring, and a 20-proton spin system was consistent with the presence of an alkyl polyamine residue. Correlations observed in HSQC and HMBC NMR experiments established that the first two proton spin systems comprised a 3-substituted indole moiety. Among these, correlations observed between resonances assigned to H-4 (δ_H 8.23) and δ_C 112.0 (C-3) and δ_C 136.2 (C-7a), between H-7 (δ_H 7.55) and δ_C 126.1 (C-3a), and between H-2 (δ_H 8.77) and C-3, C-3a, and C-7a were decisive in establishing the indole ring system. In addition, a correlation observed between H-2 and δ_C 181.6 (C-8) in the HMBC spectrum established the presence of a carbonyl group at the indole 3-position. Analysis of chemical shifts and correlations observed in COSY, HSQC, and HMBC NMR experiments for the alkyl polyamine residue determined it to represent an N^1-amidospermidine fragment (C-9 to (+)NH_3-19).[5] Interpretation of the COSY spectrum led to one contiguous polyamine substructure extending from an exchangeable proton resonance at δ_H 8.90 (t, J = 6.2 Hz, NH-10) to δ_H 3.30 (obscured by solvent, H_2-11) through H_2-12 (δ_H 1.86) and H_2-13 (δ_H 2.94) to an exchangeable protonated secondary amine resonance (δ_H 8.54, 2H), which in turn correlated to carbon-bound methylene δ_H 2.94 (H_2-15) and sequentially through δ_H 1.60 (H_2-16), δ_H 1.56 (H_2-17), and δ_H 2.80 (H_2-18) before terminating at a protonated primary amine resonance (δ_H 7.81, br s, H_3-19). HMBC correlations observed between H_2-11 (δ_H 3.30) and NH-10 (δ_H 8.90) and an amide ^{13}C resonance (δ_C 163.7, C-9) established the polyamine to be an N^1-amido-substituted spermidine unit. Between them, the 3-carbonyl-substituted indole and N^1-amidospermidine fragments accounted for all atoms required by the molecular formula, with a possible connection between C-8 and C-9 of **1**. However, no long-range

Scheme 1. Synthesis of Didemnidines A (1) and B (2)



$^{1}H-^{13}C$ NMR correlation was observed between NH-10 and C-8 to directly support the presence of this linkage. Comparison of ^{13}C chemical shift data observed for the C-2 to C-9 fragment of **1** with structurally related indole-3-glyoxylamides leptoclinidamine A[6] and polyandrocarpamide A[7] showed excellent agreement, allowing the confident assignment of the structure of didemnidine A as shown. The structure of the natural product was confirmed by synthesis, whereby coupling of 2-(indol-3-yl)glyoxylic acid (**3**)[8] with N^8-*tert*-butoxycarbonylspermidine[9,10] in the presence of PyBOP afforded N^1-(indolyl-3-glyoxamido)-N^8-*tert*-butoxycarbonylspermidine (**4**) in 47% yield (Scheme 1). Subsequent removal of the *tert*-butoxycarbonyl protecting group with TFA/CH_2Cl_2 yielded **1** as the N^{14},N^{19}-ditrifluoroacetate salt. Direct comparison of ^{1}H and ^{13}C NMR data acquired for this synthetic product with those observed for the natural product (see Supporting Information) confirmed the structure of **1**.

Didemnidine B (**2**) was isolated as a brown oil. A molecular formula of $C_{17}H_{23}BrN_4O_2$ was assigned to **2** on the basis of the

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Table 2. Antiparasitic and Cytotoxic Activities of 1, 2, 4, and 6

	IC_{50} (μM)				
	T.b.rhod.[a]	*T. cruzi*[b]	*L. don.*[c]	*P. falc.* K1[d]	L6[e]
1	59	130	>180	41	24
2	44	82	>160	15	25
4	34	88	>190	32	73
6	9.9	28	>160	8.4	25
melarsoprol[f]	0.010				
benznidazole[f]		1.35			
miltefosine[f]			0.52		
chloroquine[f]				0.20	
podophyllotoxin[f]					0.01

[a] *Trypanosoma brucei rhodesience*, STIB 900 strain, trypomastigotes stage. [b] *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage. [c] *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage. [d] *Plasmodium falciparum*, K1 strain, IEF stage. [e] L6 rat skeletal myoblast cell line. [f] Melarsoprol, benznidazole, miltefosine, chloroquine, and podophyllotoxin were used as positive controls.

pseudomolecular ion cluster observed in the (+)-HRESI mass spectrum. Close inspection of ^{1}H and ^{13}C NMR spectra and analysis of the full suite of 2D-NMR data (Table 1) established that the only differences between the natural products **1** and **2** were localized in the indole ring. Analysis of the COSY NMR spectrum indicated the presence of a 1,2,4-trisubstituted benzene ring, consistent with **2** being either a C-5- or C-6-brominated analogue of **1**. Consideration of ^{1}H NMR chemical shifts[11] as well as HMBC correlations observed between the proton resonance δ_H 8.15 (d, J = 8.4 Hz, H-4) and the quaternary aromatic carbon resonances δ_C 112.0 (C-3), δ_C 115.9 (C-6), and δ_C 137.1 (C-7a) established that didemnidine B was the 6-bromo analogue of didemnidine A. The synthesis of didemnidine B was achieved in a similar manner to that used for didemnidine A, starting this time with 2-(6-bromoindol-3-yl)-glyoxylic acid (**5**)[8,12] (Scheme 1). Deprotection of the *tert*-butoxycarbonyl-protected intermediate **6** yielded didemnidine B as the bistrifluoroacetate salt (see Supporting Information).

The occurrence of indole-3-glyoxylamides in marine organisms has been reported five times to date, with simple analogues being reported from the sponge *Spongosorites* sp., coscinamides A−C isolated from the sponge *Coscinoderma* sp., and the ascidian-derived metabolites polyandrocarpamides A−C (*Polyandrocarpa* sp.), 6-bromo-5-hydroxyindolyl-3-glyoxylate (*Syncarpa oviformis*), and leptoclinidamines A and B from *Leptoclinides durus*.[6] Of the limited number of spermidine derivatives reported from invertebrate and microbial marine organisms,[13] **1** and **2** join the ranks of squalamine[14] and analogues[15] as being the only examples of mono-N^1-substituted analogues.

Didemnidines A (**1**) and B (**2**), the *tert*-butoxycarbonyl-protected reaction intermediates **4** and **6**, and didemnimides A−D were all found to be inactive when evaluated for the ability to inhibit the superoxide respiratory burst in a model of gout and were not considered cytotoxic in testing in the Developmental Therapeutics Program, NCI. The smaller compound set of **1**, **2**, **4**, and **6** was found to be inactive in screening as phospholipase A_2 and farnesyltransferase enzyme inhibitors. Evaluation against the neglected disease parasite targets *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *Plasmodium falciparum* (K1 chloroquine-resistant strain) indicated didemnidine B (**2**) to be mildly active toward the malaria parasite (IC_{50} 15 μM), with slightly enhanced activity being detected for the more lipophilic *tert*-butoxycarbonyl-protected derivative **6** toward *P. falciparum* (IC_{50} 8.4 μM) and *Trypanosoma brucei rhodesience* (IC_{50} 9.9 μM) (Table 2). Both **2** and **6** displayed moderate cytotoxicity toward the nonmalignant L6 cell line, establishing limited selectivity toward the parasites. The ease of synthesis of **6** using a route amenable to the preparation of analogues provides the opportunity to more fully explore the structure−activity relationship of diacylated spermidines as antimalarial and antitrypanosomal agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Ultraviolet−visible spectra were run as MeOH solutions on a UV-2102 PC Shimadzu UV−vis scanning spectrophotometer. Infrared spectra were recorded using a Perkin-Elmer spectrum One Fourier-transform IR spectrometer as a dry film. NMR spectra were recorded on either a Bruker Avance DRX-600 spectrometer operating at 600 MHz for ^{1}H nuclei and 150 MHz for ^{13}C nuclei, a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei, or a Bruker Avance DRX-300 spectrometer operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.43; CD_3OD: δ_H 3.30, δ_C 49.05; $CDCl_3$: δ_H 7.25, δ_C 77.0). Standard Bruker pulse sequences were utilized. HRMS data were acquired on either a VG-7070 or a Bruker micrOTOF Q II mass spectrometer. Flash column chromatography was performed using reversed-phase Merck Lichroprep RP-8 or RP-18 40−63 μm, and size exclusion chromatography on Pharmacia Biotech Sephadex LH-20. Analytical reversed-phase HPLC was run on a Waters 600 HPLC photodiode array system using an Alltech C_8 column (3 μm Econosphere Rocket, 33 × 7 mm) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min and monitoring at 330 nm.

Animal Material. The ascidian was collected by scuba at a depth of 7 m from wooden piles at Tiwai Wharf, Tiwai Point, Southland, New Zealand (46°35.52′ S, 168°21.14′ E) on August 20, 2007, and kept frozen until used. A voucher specimen of the orange, encrusting irregular shaped ascidian *Didemnum* sp. is held at the National Institute for Water and Atmospheric Research, Private Bag 14-901, Kilbirnie, Wellington, New Zealand, as MNP9167. A taxonomic description and in situ underwater photograph of the organism are included in the Supporting Information.

Isolation and Purification. A single specimen of freeze-dried ascidian material (29.3 g) was extracted with MeOH (7 × 200 mL). The solvent was filtered and then removed under reduced pressure to give a dark brown extract (3.29 g). This extract was subjected to combinations of reversed-phase C_8 flash, C_{18} flash (MeOH, H_2O−TFA (0.05%)), and Sephadex LH-20 (MeOH, 0.05% TFA) chromatography, yielding didemnimide A (6.2 mg, 0.02% dry weight, t_R 4.77 min), didemnimide B (4.8 mg, 0.02% dry weight, t_R 4.75 min), didemnimide C (8.3 mg, 0.03% dry weight, t_R 4.82 min), didemnimide D (12.1 mg, 0.04% dry weight, t_R 4.74 min), didemnidine B (**2**) (2.0 mg, 0.007% dry weight, t_R 5.45 min), and didemnidine A (**1**) (1.1 mg, 0.004% dry weight, t_R 5.65 min).

*Didemnidine A (**1**):* brown oil; UV (MeOH) λ_{max} (log ε) 255 (3.49), 266 (3.42), 275 sh (3.35), 328 (3.04) nm; ^{1}H and ^{13}C NMR (see Table 1); (+)-ESIMS m/z 317 $[M + H]^+$; (+)-HRESIMS m/z 317.1980 $[M + H]^+$ (calcd for $C_{17}H_{25}N_4O_2$, 317.1972).

*Didemnidine B (**2**):* brown oil; UV (MeOH) λ_{max} (log ε) 259 (4.05), 276 (4.04), 325 (3.90) nm; ^{1}H and ^{13}C NMR (see Table 1); (+)-ESIMS m/z 395 ($[M + H]^+$, 50%), 397 ($[M + H]^+$, 50%); (+)-HRESIMS $m/$

z 395.1075 [M+H]$^+$ (calcd for $C_{17}H_{24}{}^{79}BrN_4O_2$, 395.1077), 397.1054 (calcd for $C_{17}H_{24}{}^{81}BrN_4O_2$, 397.1057).

*N^1-(Indolyl-3-glyoxamido)-N^8-tert-butoxycarbonylspermidine (**4**).* N^8-*tert*-Butoxycarbonyl spermidine[9,10] (18.1 mg, 73.4 μmol), 2-(indol-3-yl)glyoxylic acid (**3**)[8] (11.6 mg, 61.2 μmol), and PyBOP (35.0 mg, 67.1 μmol) were dissolved in dry DMF (10 mL), triethylamine (34.1 μL) was added, and the reaction was left to stir at room temperature for 24 h. The crude reaction mixture was subjected to combinations of C_8, C_{18}, and Sephadex LH-20 (MeOH, H_2O−TFA (0.05%)) column chromatography to afford N^1-(indolyl-3-glyoxamido)-N^8-*tert*-butoxycarbonylspermidine trifluoroacetate salt (**4**) (15.3 mg, 47%) as a pale yellow oil: IR (neat) ν_{max} 3389, 1677, 1437, 1204, 1136 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.79 (1H, s, H-2), 8.29 (1H, m, H-4), 7.47 (1H, m, H-7), 7.26 (2H, m, H-5 and H-6), 3.46 (2H, m, H_2-11), 3.05 (6H, m, H_2-13, H_2-15, H_2-18), 1.98 (2H, m, H_2-12), 1.71 (2H, m, H_2-16), 1.56 (2H, m, H_2-17), 1.41 (9H, s, OBu^t); ^{13}C NMR (CD_3OD, 100 MHz) δ 181.5 (C-8), 166.5 (C-9), 158.7 (C-1′), 139.6 (C-2), 138.0 (C-7a), 128.0 (C-3a), 125.0 (C-6), 124.0 (C-5), 123.0 (C-4), 114.0 (C-3), 113.2 (C-7), 80.2 (C-2′), 48.4 (C-15, obscured by solvent), 46.6 (C-13), 40.5 (C-18), 36.9 (C-11), 28.8 (OBu^t), 28.1 (C-17), 27.6 (C-12), 24.6 (C-16); (+)-ESIMS m/z 417 [M + H]$^+$; (+)-HRESIMS m/z 417.2509 [M + H]$^+$ (calcd for $C_{22}H_{33}N_4O_4$, 417.2496).

*Didemnidine A (**1**).* To a solution of **4** (10.1 mg, 19.1 μmol) in CH_2Cl_2 (20 mL) was added TFA (10 mL). The mixture was stirred at room temperature for 70 min, solvent was removed under reduced pressure, and the crude product was purified by Sephadex LH20 column chromatography (MeOH + 0.05% TFA) to yield didemnidine A (**1**) as the bistrifluoroacetate salt (7.2 mg, 70%, yellow oil): IR (neat) ν_{max} 3351, 1672, 1633, 1495, 1436, 1200, 1141 cm^{-1}; UV, ^{1}H and ^{13}C NMR data were identical to those observed for the isolated natural product; (+)-ESIMS m/z 317 [M + H]$^+$; (+)-HRESIMS m/z 317.1973 [M + H]$^+$ (calcd for $C_{17}H_{25}N_4O_2$, 317.1972).

*2-(6-Bromoindol-3-yl)glyoxylic acid (**5**).* 6-Bromoindole[12] (0.535 g, 2.72 mmol) in a 100 mL two-necked round-bottomed flask was dissolved in anhydrous ether (30 mL). A solution of oxalyl chloride (2.72 mmol) in anhydrous ether (10 mL) was added dropwise to the rapidly stirring indole solution at 0 °C under a nitrogen atmosphere over a period of 10 min. The mixture was stirred for an additional 10 min, and an orange precipitate formed. Saturated $NaHCO_3$ (10 mL) was added dropwise to the solution and the mixture then heated to reflux for 30 min. Upon cooling, the reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL) and then acidified with 10% HCl(aq) (10 mL) to yield **5** as a yellow precipitate, which was then filtered and dried (0.650 g, 89%): mp 233−234 °C (dec); IR (neat) ν_{max} 3203, 1712, 1634, 1512, 1410, 1269 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.63 (1H, s, NH), 8.38 (1H, d, J = 3.1 Hz, H-2), 8.05 (1H, d, J = 8.7 Hz, H-4), 7.76 (1H, d, J = 1.4 Hz, H-7), 7.37 (1H, dd, J = 8.7, 1.4 Hz, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.1 (C-8), 165.2 (C-9), 138.9 (C-2), 137.8 (C-7a), 125.9 (C-5), 124.8 (C-3a), 123.0 (C-4), 116.5 (C-6), 115.8 (C-7), 112.5 (C-3); (−)-ESIMS m/z 266 [M − H]$^-$ (50%), 268 [M − H]$^-$ (50%); (−)-HRESIMS m/z 265.9460 [M − H]$^-$ (calcd for $C_{10}H_5{}^{79}BrNO_3$, 265.9458), 267.9443 (calcd for $C_{10}H_5{}^{81}BrNO_3$, 267.9438).

*N^1-(6-Bromoindolyl-3-glyoxamido)-N^8-tert-butoxycarbonylspermidine (**6**).* N^8-*tert*-Butoxy carbonylspermidine[9,10] (22.0 mg, 89.7 μmol), 2-(6-bromoindol-3-yl)glyoxylic acid (**5**) (20.1 mg, 75.3 μmol), and PyBOP (40.0 mg, 76.8 μmol) were dissolved in dry DMF (10 mL), triethylamine (40 μL) was added, and the reaction was left to stir at room temperature for 24 h. The crude reaction mixture was subjected to combinations of C_8 flash, C_{18} flash, and Sephadex LH-20 (MeOH, H_2O−TFA (0.05%)) column chromatography to afford N^1-(6-bromoindolyl-3-glyoxamido)-N^8-*tert*-butoxycarbonylspermidine trifluoroacetate (**6**) (18.2 mg, 40%) as a pale yellow oil: IR (neat) ν_{max} 3388, 1675, 1460, 1205, 1131 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.79 (1H, s, H-2), 8.20 (1H, d, J = 8.3 Hz, H-4), 7.66 (1H, d, J = 1.6 Hz, H-7), 7.36 (1H, dd, J = 8.3, 1.6 Hz, H-5), 3.45 (2H, m, H_2-11), 3.05 (6H, m, H_2-13, H_2-15, H_2-18), 1.98 (2H, m, H_2-12), 1.70 (2H, m, H_2-16), 1.55 (2H, m, H_2-17), 1.41 (9H, s, OBu^t); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.1 (C-8), 166.0 (C-9), 158.7 (C-1′), 140.1 (C-2), 138.8 (C-7a), 127.0 (C-5), 126.9 (C-3a), 124.4 (C-4), 118.1 (C-6), 116.2 (C-7), 114.0 (C-3), 80.1 (C-2′), 48.8 (C-15), 46.5 (C-13), 40.5 (C-18), 36.9 (C-11), 28.7 (OBu^t), 28.1 (C-17), 27.5 (C-12), 24.6 (C-16); (+)-ESIMS m/z 495 [M + H]$^+$ (50%), 497 [M + H]$^+$ (50%); (+)-HRESIMS m/z 495.1597 [M + H]$^+$ (calcd for $C_{22}H_{32}{}^{79}BrN_4O_4$, 495.1601), 497.1579 (calcd for $C_{22}H_{32}{}^{81}BrN_4O_4$, 497.1582).

*Didemnidine B (**2**).* To a solution of **6** (9.8 mg, 16.1 μmol) in CH_2Cl_2 (20 mL) was added TFA (10 mL). The mixture was stirred at room temperature for 70 min, solvent was removed under reduced pressure, and the crude product was purified by Sephadex LH20 column chromatography (MeOH + 0.05% TFA) to yield didemnidine B (**2**) as the bistrifluoroacetate salt (7.0 mg, 70%, yellow oil): IR (neat) ν_{max} 3254, 2954, 1672, 1605, 1504, 1125 cm^{-1}; UV, ^{1}H and ^{13}C NMR data were identical to those observed for the isolated natural product; (+)-HRESIMS m/z 395.1069 [M + H]$^+$ (calcd for $C_{17}H_{24}{}^{79}BrN_4O_2$, 395.1077), 397.1049 (calcd for $C_{17}H_{24}{}^{81}BrN_4O_2$, 397.1057).

Biological Assays. Details of the neutrophil anti-inflammatory assay,[16] PLA_2 and farnesyltransferase assays,[17] and whole organism parasite assay protocols[18] have been reported elsewhere.

■ ASSOCIATED CONTENT

Supporting Information. Taxonomic description and color in situ photo of the *Didemnum* sp. ascidian, assigned ^{1}H and ^{13}C NMR data (DMSO-d_6) for didemnimides A−D as their trifluoroacetate salts, ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectra (DMSO-d_6) for didemnidines A (**1**) and B (**2**), ^{1}H NMR spectra of synthetic intermediates **4** and **6**, and ^{1}H and ^{13}C NMR spectra for synthetic **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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