N,N′-Methyleno-didemnin A from the Ascidian *Trididemnum solidum*. Complete NMR Assignments and Confirmation of the Imidazolidinone Ring by Strategic Analysis of $^1J_{CH}$

Tadeusz F. Molinski,*[,†,‡] Jaeyoung Ko,[†] Kirk A. Reynolds,[†] Sarah C. Lievens,[§] and Katrina R. Skarda[§]

[†]Department of Chemistry and Biochemistry, and [‡]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive MC0358, La Jolla, California 92093-0358, United States

[§]Department of Chemistry, University of California, Davis, 1 Shields Avenue, Davis, California 95616, United States

S *Supporting Information*

ABSTRACT: The complete NMR assignments of N,N′-methyleno-didemnin A from *Trididemnum solidum* Van Name 1902 are reported along with a strategic analysis of $^1J_{CH}$ coupling constants that confirm the presence of the imidazolidinone ring.
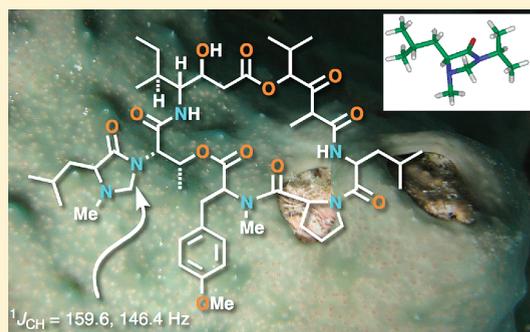
The Caribbean ascidian *Trididemnum solidum* Van Name 1902 is the source of the highly cytotoxic compounds didemnins (Figure 1), which have been advanced to human clinical trials as potential anticancer agents.[1,2] The first described members of the series, didemnins A (**1**) and B (**2**),[1] are cyclic depsipeptides containing the uncommon amino acids statine and N,O-dimethyltyrosine.[3,4] Advanced clinical trials of didemnin B (**2**) for treatment of various solid tumors[5] have been discontinued; however, dehydro-didemnin B (**3**, aplidine) has been launched into phase II clinical trials. Despite considerable investigations, the mechanism of action of didemnin B and its induction of apoptosis in cancer cells is not completely understood. Our collections of *T. solidum* from the Bahamas gave extracts—not unexpectedly—that displayed strong cytotoxicity against cultured human colon tumor cells (HCT-116) due to the presence of **1**, **2**, and their congeners. We describe here the complete NMR characterization of N,N′-methyleno-didemnin A (**4**), a derivative of didemnin A that co-occurs with **1** and **2** in *T. solidum* in which the terminal N-methyl leucyl residue has undergone cyclization to a substituted N-methyl 1-methylimidazolidin-4-one.[6] Interpretation of the scalar heteronuclear coupling constants of **4** and three synthetic 1-methylimidazolidin-4-ones reveals useful signature values of $^1J_{CH}$ values that confirm the identity of the heterocyclic ring.

T. solidum was exhaustively extracted with MeOH, and the concentrated extract (IC$_{50}$ <38 ng/mL against cultured human colon cancer cells, HCT-116) was triturated with anhydrous MeOH and CHCl$_3$ to remove salts. The concentrated MeOH-soluble
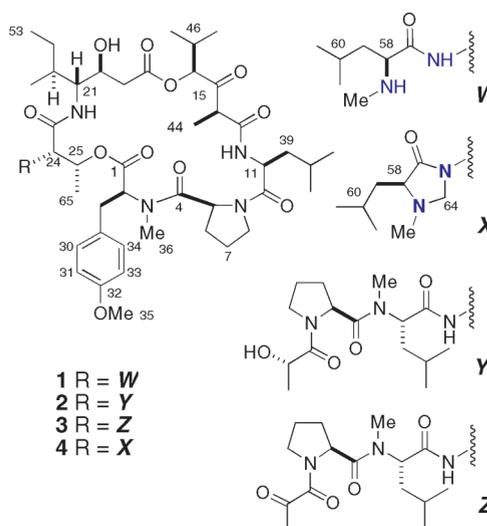
Figure 1. Structures of didemnins A (**1**) and B (**2**), aplidine (dehydro-didemnin B, **3**), and N,N′-methyleno-didemnin A (**4**).

portion was fractionated by Sephadex LH-20 size exclusion chromatography eluting with MeOH and finally purified by reversed-phase HPLC to provide **4** along with didemnins A

(**1**) and B (**2**), which were identified by comparison of MS and ^{1}H NMR data (C_6D_6) with literature data.[7] Compound **4** was isolated as a clear glass (HRMS $[M + H]^+ = 955.5733$) with a formula $C_{50}H_{78}N_6O_{12}$, corresponding to the addition of one C atom to **1**, $C_{49}H_{78}N_6O_{12}$. Due to severe signal overlap of signals of **4** in CD_3OD, the ^{1}H NMR spectrum was rerecorded in several other solvents, including $CDCl_3$, C_6D_6, C_5D_5N, and $(CD_3)_2SO$. The best dispersion of NMR signals for **4** was achieved in C_6D_6, which also permitted the observation and correlation of the exchangeable NH and OH protons. The ^{1}H NMR data of **4** (C_6D_6, Table 1) were highly suggestive of a peptide of the didemnin class with spin systems corresponding to an approximated A_2B_2 pattern for a *para*-substituted phenolic ring (δ_H 6.78, d, $J = 8.4$ Hz. δ_C 130.4, d; 6.70, d, $J = 8.4$ Hz. δ_C113.6), two O-CH, eight CH attached to electronegative substituents, two secondary amide protons, (C=O)NH, a hydroxy proton, nine methyl doublets, one methyl triplet, two *N*-methyl singlets, and an *O*-methyl singlet. These structural features were readily correlated with spin systems found in **1**, except for two changes: amine N-H and one amide-N-H were replaced by a downfield shifted CH_2 group (δ_H 5.05, d, $J = -4.2$ Hz; 3.35, dd, $J = -4.2$, 1.2 Hz; δ_C 68.9, d).[8] The H-64α and H-64β diastereotopic signals were correlated by COSY cross-peaks to H-58[9] and HMBC cross-peaks to C-63 and C-58. Taken together, the signals of **4** could be accommodated by placement of the protons within an imidazolidinone ring. Finally, compound **4**, prepared from **1** (paraformaldehyde, benzene, 80 °C), was shown to be identical with the sample isolated from *T. solidum* (^{1}H NMR and MS). The structure of **4** was first proposed by Gloer;[10] however, incomplete MS and NMR data allowed only a tentative assignment.

While the above deductive reasoning supported the presence of an imidazolidinone ring system in **4**, we sought independent spectroscopic evidence in the form of $^1J_{CH}$ coupling constants. The unique feature of the heterocyclic ring is two N atoms bonded to a sp^3-hybridized CH_2 group. The magnitude of $^1J_{CH}$ in sp^3 C-C-H couplets changes with the replacement of C with electronegative substituents and can be used to identify the attached atoms. For example, the $^1J_{CH}$ was used to discriminate two possible constitutional isomers of varamines A and B and supports the presence of an *S*-Me over an alternative *N*-Me group.[11] The one-bond heteronuclear coupling constant $^1J_{CH}$ is known to be primarily dependent upon C hybridization (% s character of the associated molecular orbitals), electronegativity of attached atoms, the presence of electron-withdrawing groups, stereoelectronic effects, and, in the case of cyclic structures, ring strain. Values for $^1J_{CH}$ in unstrained alkanes (R$_3$C-H) are essentially invariant within a narrow range of 125–130 Hz. Substitution of the C—H couplet with an N-substituent increases the magnitude of $^1J_{CH}$ to 135–140 Hz, while the greater electronegativity of O-substituents further increases $^1J_{CH}$ to 145–150 Hz. The bonding of two electronegative atoms to C-H (e.g., O-CH-O, N-CH-O, and N-CH-N groups) raises the value of $^1J_{CH}$ over their monosubstituted analogues. The best studied cases of $^1J_{CH}$ include the anomeric O-CH-O groups in aldoses and glycosides, which show values of O-CH-O in the ranges $^1J_{CH} = 169$–171 and 158–162 Hz for axial and equatorial substituents, respectively.[12]

The values of $^1J_{CH}$ in **4** were conveniently obtained from ^{13}C-coupled HSQC spectra (measured with the ^{13}C decoupler turned off during FID acquisition; see Table 2). ^{13}C-coupled cross-peaks were observed for the AB system corresponding to the C-64 CH_2 group of **4** revealing two different couplings ($^1J_{CH} = 159.6$ and 146.4 Hz) for the diastereotopic H-64α and H-64β protons (Table 2), respectively. In comparison, *N*-CH_3 groups showed lower scalar coupling values (e.g., $^1J_{CH}$ (C-63) = 135.0 Hz; (C-36) = 138.6 Hz), while α-CH signals show characteristic couplings (e.g., $^1J_{CH}$ (C-11) = 148.2 Hz). Clearly, C-64 is substituted with two N atoms. It is of interest to note that $^1J_{CH}$ of the diastereotopic C-64 geminal C—H couplets are not equal and that subtle stereoelectronic effects also influence the magnitude of the heteronuclear coupling, albeit with an attenuated effect ($\Delta J \approx 5\%$ of the value of $^1J_{CH}$).

Finally, we prepared the model compounds **5**–**8** (Scheme 1) and compared the measured $^1J_{CH}$ values with those of **4**. Coupling (EDCI) of (*S*)-*N*-methyl-*N*-Boc-leucine, obtained by hydrolysis of the corresponding methyl ester **9**,[13] with phenethylamine or isopropylamine followed by deprotection (TFA) and treatment of the amide products with paraformaldehyde in benzene gave the *N*-methylimidazolidin-4-ones **5** and **6**, respectively. Ammoniolysis of a mixed anhydride derived from **9** gave the corresponding leucinamide, which was converted through a similar sequence to **7**; an *N*-hydroxymethyl imidazolidinone formed by addition of a second formaldehyde equivalent to the amide nitrogen. Finally, pseudoephedrine was converted to the known oxazolidine **8**[14] in a similar manner. The coupled HSQC spectra of **5**–**7** showed scalar heteronuclear couplings for the AB signals[15] corresponding to the diastereotopic N-CH_2-N protons ($^1J_{CH} = 154.3$, 144.4 Hz; 154.4, 143.8 Hz, 157.1, and 145.5 Hz, respectively). Notably, the second ^{1}H NMR AB spin system in **7**, corresponding to the N-CH_2-OH group, showed slightly larger and almost equivalent heteronuclear scalar couplings ($^1J_{CH} = 157.1$, 156.7 Hz), as would be expected for an aminal group that replaces an N atom for a more electronegative O. Larger coupling constants were found for the diastereotopic N-CH_2-*O* group in oxazolidine **8** ($^1J_{CH} = 159.6$, 151.7 Hz), which lacks an electron-withdrawing *N*-C=O group. In contrast, carbons substituted with a single N atom have a much lower heteronuclear coupling constant; for **5**–**8** the values for *N*-CH_3 groups fell into the narrow range $^1J_{CH} = 133.5$–135.5 Hz. The differential values of $^1J_{CH}$ in the diastereotopic N-CH_2-N groups seen in **5**–**7** were entirely comparable to those of **4** and consistent with the depicted structure. These observations may be put to further practical use, not only in elimination of carbinolamines as possible structures for **4**[16] but for differentiation of 2-unsubstituted oxazolidines from imidazolidinones. Given the sensitivity of modern microcryoprobe NMR spectrometers, measurement of $^1J_{CH}$ values by ^{13}C-coupled [1]H—^{13}C HSQC is practical, even with only microgram amounts of natural products.[17]

In order to gain insight into the assignment of the two well-dispersed N-CH_2-N' proton signals in **4**–**7**, molecular mechanics calculations (MMFF) were carried out on **6** to ascertain the preferred conformations. Minimized structures were found ($N = 25$), and Monte Carlo searching revealed that the lowest four conformers accounted for 73% of the conformer distribution. Figure 2 depicts models of the two lowest conformers with energies and Boltzmann weightings of −1.8 kcal mol⁻¹ (36.3%) and −0.56 kcal mol⁻¹ (21.3%). The imidazolidinone is relatively flat except for the *N*-Me that lies below the plane of the ring *syn* to Hα in order to reduce nonbonded interactions. It is likely that, of the two diastereotopic protons on the N-CH_2-N' group, the proton *syn*-facial to Hα 4 in the imidazolidinone participates in the consistently observed long-range $^4J_{HH}$ in the ^{1}H NMR spectra of **4**–**7** as the two H's and intervening bonds conform
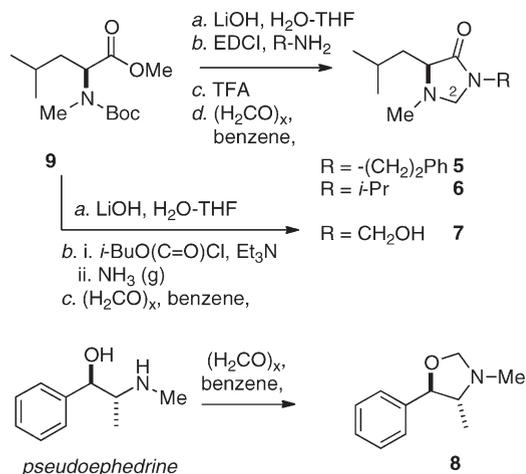
Table 1. NMR Assignments of *N,N′*-Methyleno-didemnin A (4) (600 MHz, C_6D_6)

no.	$\delta_C^{a,b}$	δ_H^b, mult., J (Hz)	δ_N^c	COSY^b	HMBC (#C)^b
1	168.4				
2	65.9	3.13, dd (11.3, 4.5)		28α, 28β	36
4	169.7^d				36
5	57.3	4.33, dd (8.7, 5.1)		6β	
6α	22.8	1.07, m		7α, 8β	7
6β		1.28, m		5, 7β	
7α	24.1	1.00, m		6β, 7β, 8α, 8β	5
7β		1.40, m		7α, 8α, 8β	
8α	46.5	2.95, ddd (−13.2, 9.6, 2.4)		6α, 7α, 7β	5
8β		3.27, ddd (−13.2, 9.0, 2.4)		6α, 7α, 7β	7
10	170.7^d				39
11	50.0	5.16, td (9.0, 2.7)		12, 39, 40	
12N		8.61, d (9.0)	119.2	11	13
13	170.1				
14	50.1	4.51, q (6.2)		44	13, 15, 44
15	204.3				
16	80.9	5.79, d (3.0)		46	15, 18, 46
18	172.7				
19α	39.3	2.60, dd (−17.6, 10.2)		19β, 20	18, 20
19β		3.65, d (−17.6)		19α, 21	18
20	67.3	4.37, td (10.2, 2.0)		19α, 21, 50	
21	54.4	4.54, td (10.2, 3.0)		19β, 20, 22, 51	20, 54
22N		7.65, d (10.0)	117.4		
23	169.0				
24	57.5	5.32, d (2.3)			1, 23, 25, 65
25	69.9	5.42, qd (6.4, 2.3)		65	23, 24
28α	33.8	3.46, dd (−14.1, 4.5)		2, 28β	2
28β		3.56, dd (−14.1, 11.3)		2, 28α	2, 29
29	130.0				
30,34	130.4	6.78, d (8.4)		31/33	29,32
31,33	113.6	6.70, d (8.4)		30/34	34,
32	158.8				
35	54.4	3.33, s			32
36	38.1	2.04, s			2
39α	42.2	1.85, m		11, 40	
39β		1.85, m		11, 40	
40	24.9	1.92, m		11, 39, 41, 42	
41	23.6	0.88, d (6.6)		40	39, 40, 42
42	21.0	1.08, d (6.0)		40	39, 40, 41
44	15.0	1.77, d (6.2)		14	13, 14, 15
46	30.6	2.49, hep.d (7.2, 3.0)		16, 47, 48	
47	16.4	0.84, d (7.2)		46	16, 46, 48
48	18.8	0.75, d (7.2)		46	16, 46, 47
50 OH		2.95, d (2.0)		20	
51	34.0	2.35, hex.d (6.6, 3.0)		21, 52α, 52β, 54	
52α	27.7	1.40, m		51, 52β	21, 51
52β		1.69, m		51, 52α, 53	51, 53, 54
53	13.0	1.18, t (7.2)		52β	51, 52
54	14.6	1.03, d (7.2)		51	21, 51, 52
57	174.4				
58	64.3	2.77, t (5.7)		59α, 59β, 64α, 64β	57
59α	37.8	1.57, ddd (13.2, 8.4, 4.8)		58, 59β, 60	57, 58
59β		1.69, m		58, 59α, 60	57, 58
60	24.4	2.07, m		59α, 59β, 61, 62	59

Table 1. Continued

no.	$\delta_C^{a,b}$	δ_H^{b}, mult., J (Hz)	δ_N^{c}	COSY[b]	HMBC (#C)[b]
61	23.2	0.93, d (6.6)		60	59, 60, 62
62	22.4	0.97, d (6.6)		60	59, 60, 61
63	40.0	1.96, s			59, 64
64α	68.9	3.35, dd (−4.2, 1.2)		58, 64β	63
64β		5.05, d (−4.2)		58, 64α	
65	17.9	1.45, d (6.4)		25	25

[a] Chemical shifts from HSQC, HMBC cross-peaks (600 MHz), room-temperature probe. [b] Spectra referenced to C_6D_5H (δ_H 7.16) and C_6D_6 (δ_C 128.06). [c] Measured from $^1H-^{15}N$ HSQC cross-peaks. [d] gHMBC ($^{2,3}J$ = 8.4 Hz), cryomicroprobe.

Scheme 1. Syntheses of Model Compounds 5−8[a]

[a] See Table 2 for $^1J_{CH}$ values.

Figure 2. Molecular mechanics minimized structures for **6** (MMFF, Spartan 08) and Boltzman-weighted energies: (a) $E = -1.8$ kcal mol^{-1} (36.3%); (b) $E = -0.56$ kcal mol^{-1} (21.3%).

to an approximate "W" conformation, although this must be considered only a tentative assignment. No useful NOEs were observed in the NMR spectra of **4** that would otherwise clarify this assignment.
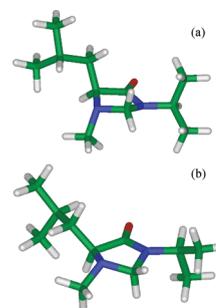
The cytotoxicity of **1** and **4** toward cultured human colon tumor cell lines (HCT-116) was briefly evaluated. N,N'-Methyleno-didemnin A (**4**) was found to be slightly more active (IC$_{50}$ 24 nM) than didemnin A (**1**; IC$_{50}$ 32 nM), but less so than the very potent didemnin B (**2**; IC$_{50}$ 9.0 nM).

The higher didemnins have only been partially characterized. Although the closely related formamide didemnin G (N^α-formyl-didemnin A) and didemnin X,[1b] a higher molecular mass imidazolidinone, were isolated from *T. solidum*, compound **4** was known previously as a condensation product of formaldehyde with didemnin A (**1**).[10] Here, we provide the first complete structural characterization of **4** and confirm its occurrence as a natural product in *T. solidum* from the Bahamas.[10,18]

In summary, we have isolated and completely assigned the NMR spectra of **4**, an N,N'-methyleno derivative of didemnin A from *T. solidum* that shows similar cytotoxicity to didemnin A (**1**) against HCT-116 cells. A useful method, based on $^1J_{CH}$ values, to differentiate oxazolidinones, carbinolamines, and imidazolidinone-modified peptides was validated by critical comparison with synthetic model compounds.

■ EXPERIMENTAL SECTION

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded in CDCl$_3$ on several instruments: a Bruker DRX 600 MHz instrument, equipped with a 1.7 mm $\{^{13}C,^{15}N\}^1H$ CPTXI microcryoprobe, a Jeol ECA 500 MHz spectrometer equipped with a 5 mm $\{^{13}C\}^1H$ probe, or a Varian Mercury 400 MHz instrument equipped with a 5 mm $\{^1H\}^{13}C$ probe and measured at 23 °C in CDCl$_3$ or C_6D_6 with reference to residual solvent signals (CDCl$_3$ ^1H, δ 7.26 ppm; ^{13}C, δ 77.16 ppm; C_6D_6 ^1H, δ 7.15 ppm; ^{13}C, δ 128.1 ppm). HRMS measurements were measured by MALDI (UC Riverside) or ESI conditions with an Agilent 6000 series TOF mass spectrometer (Scripps Research Institute MS facility) or a ThermoFinnigan Orbitrap mass spectrometer (UC San Diego, Small Molecule MS facility). Semipreparative HPLC was carried out with a Varian high-capacity dual-pump system coupled to a Rainin UV-1 high-dynamic UV detector under specified conditions. All solvents for HPLC purification were of commercial HPLC grade.

Animal Material. *Trididemnum solidum* (99-09-048) was collected by hand using scuba from Little San Salvador, Bahamas, in 1999 at a depth of 20 m and stored frozen until extracted. A second collection (07-35-213) was made in the same area in 2008. The specimen consisted of thick, tough-to-tear sheets that were pale green on the exposed surface and pale pinkish on the attached underside due to the presence of cyanobacterial phycoerythrin. The surface of this colonial ascidian was very finely textured with darker colored zooids embedded within a pale green tunic. Additionally, the MeOH extract of the animal was deeply pigmented with chlorophyll, a signature property for the presence of cyanobacteria. Together with other morphological features, these traits are uniquely associated with the common *T. solidum* of the Caribbean Sea. A preserved type sample is archived at the University of California, San Diego.

Extraction and Isolation. The tunicate (402.3 g, frozen) was mascerated and extracted four times with MeOH (total of 6 L). The combined extracts were concentrated under reduced pressure and the green-white solids triturated with anhydrous MeOH, followed by CHCl$_3$. The deep-green MeOH-soluble fraction was concentrated under reduced pressure before separation by size exclusion chromatography over a column of Sephadex LH-20 eluting with MeOH. The didemnin-containing fraction was further purified by reversed-phase

Table 2. Selected $^1J_{CH}$ for 4, Imidazolidinones 5−7, and Oxazolidine 8

cmpd	#H[a]	#C	$^1J_{CH}$[b]/Hz	group
4	11	11	148.2	NH-CHR-C(O)
4	20	20	147.9	R_2-CH-O
4	21	21	144.6	R_2-CH-NHC(O)
4	31	31	156.6	Ph
4	36	36	138.6	N-CH$_3$
4	41	41	124.8	R-CH$_3$
4	51	51	129.6	R-CH$_3$
4	63	63	135.0	N-CH$_3$
4	59α,β	59	123.0, 128.4	R-CH$_2$-R′
4	64α,β	64	159.6, 146.4	Nα-CH$_2$−NHC(O)
5	2α,β	2	154.3, 144.5	Nα-CH$_2$-NHC(O)
6	2α,β	2	154.4, 143.8	Nα-CH$_2$-NHC(O)
7	2α,β	2	157.1, 145.2	Nα-CH$_2$-NHC(O)
7			157.1, 156.7	NH-CH$_2$OH
8			159.6, 151.7	N-CH$_2$-O
5			135.5	Nα-Me
6			133.5	Nα-Me
7			134.8	Nα-Me
8			134.0	N-Me

[a] Note different locant numberings; see Figure 1 and Scheme 1. [b] $^1J_{CH}$ measured from ^{13}C-coupled HSQC spectra (600 MHz, C$_6$D$_6$).

HPLC eluting with 85:15 MeOH/H$_2$O to give didemnin B (2) (86.7 mg, 0.022% wet weight) and $N,N′$-methyleno-didemnin A (4) (6.83 mg, 0.0017% wet weight). The second collection (07-35-213) was processed in a different manner. The frozen tunicate (56.3 wet wt) was homogenized with MeOH (2 × 400 mL) using a hand blender, and the combined deep-green aqueous MeOH extracts were partitioned sequentially against hexanes, CHCl$_3$, and n-BuOH. The CHCl$_3$-soluble fraction was separated by silica gel chromatography (MeOH/CHCl$_3$, stepped gradient), and the didemnin-containing fraction subjected to HPLC (Phenomenex Luna C$_{18}$, 10 × 250 mm, 85:15 MeOH/H$_2$O) to give didemnin A (1, 8.63 mg, 0.015% w/w wet wt).[1]

***N,N′*-Methyleno-didemnin A (4):.** colorless glass; $[\alpha]_D$ −153 (c 0.38, MeOH); ^{1}H NMR, ^{13}C NMR, see Table 1; ^{13}C-coupled HSQC, see Table 2; MALDI-HRMS m/z 955.5733 [M + H]$^+$ (calcd for C$_{50}$H$_{79}$N$_6$O$_{12}$, 955.5755).

Synthesis of *N,N′*-Methyleno-didemnin A (4) from Didemnin A (1). Compound 4 was prepared from didemnin A (1) using a variant of Gloer's method.[10] A solution of 1 (2.18 mg, 2.31 μmol) in benzene (2 mL) was treated with excess paraformaldehyde (0.71 mg). The mixture was heated at 80 °C (1.5 h) until LC-MS indicated consumption of starting material. The benzene layer was washed with aqueous K$_2$CO$_3$ (10% w/v), dried over solid K$_2$CO$_3$, and concentrated under a stream of N$_2$. Separation of the crude product by HPLC (C$_{18}$ reversed phase, 85:15 MeOH/H$_2$O) gave pure 4 (0.45 mg), identical with the natural product (^{1}H NMR, MS). Without alkali workup, product 4 was unstable to the conditions of purification.

(*S*)-5-Isobutyl-1-methyl-3-phenethylimidazolidin-4-one (5). A mixture of (*S*)-N-Boc-N-methylleucine (200 mg, 0.82 mmol, prepared by saponification of the corresponding methyl ester 9[13]) and EDCI (236 mg, 1.23 mmol) in THF (5 mL) was stirred for 15 min, then treated with phenethylamine (149 mg, 1.23 mmol), then stirred for an additional 8 h before diluting with H$_2$O and extraction with EtOAc (×3). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure, and the residue was purified by flash chromatography (SiO$_2$, 1:20 EtOAc/hexane) to give the corresponding (*S*)-phenethylamide (121 mg, 59%). The crude product (60 mg) was

taken up in CH$_2$Cl$_2$ (0.5 mL) and stirred in the presence of TFA (0.5 mL) until TLC indicated the complete deprotection to the corresponding secondary amine TFA salt (ninhydrin). The crude product was suspended in benzene containing excess paraformaldehyde (52 mg) and heated at 80 °C for 4 h. After cooling, the solution was washed sequentially with aqueous NaHCO$_3$ and brine, then dried over Na$_2$SO$_4$. The mixture was concentrated under reduced pressure and purified by flash chromatography (SiO$_2$, 1:2 EtOAc/hexane) to afford 5 (24 mg, 53%): $[\alpha]_D$ +24.5 (c 0.01, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.24 (m, 2H), 7.17 (m, 3H), 4.17 (d, 1H, J = 5.2 Hz), 3.55 (dd, 1H, J = 5.2, 1.5 Hz), 3.49 (m, 2H), 2.80 (m, 3H), 2.32 (s, 3H), 1.85 (m, 1H), 1.53 (m, 1H), 1.36 (m, 1H), 0.90 (d, 3H, J = 6.8 Hz), 0.88 (d, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.8, 138.6, 128.8, 128.7, 126.7, 70.6, 65.2, 42.7, 41.1, 39.5, 34.2, 24.9, 23.1, 22.7; ^{13}C-coupled HSQC, see Table 2; HRESIMS m/z 261.1961 [M + H]$^+$ (calcd for C$_{16}$H$_{25}$N$_2$O, 261.1967).

(*S*)-5-Isobutyl-1-methyl-3-isopropylimidazolidin-4-one (6). Compound (*S*)-6 was prepared using the above procedure. (*S*)-6: $[\alpha]_D$ +26 (c 0.008, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 4.34 (dd, 1H, J = 5.3, 0.8 Hz), 4.29 (m, 1H), 3.68 (dd, 1H, J = 5.3, 1.7 Hz), 2.86 (m, 1H), 2.43 (s, 3H), 1.92 (m, 1H), 1.61 (m, 1H), 1.41 (m, 1H), 1.53 (d, 3H, J = 6.8 Hz), 1.14 (d, 3H, J = 6.8 Hz), 0.94 (d, 3H, J = 6.8 Hz), 0.92 (d, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.8, 65.8, 65.7, 42.3, 40.9, 39.4, 25.0, 23.0, 22.8, 20.2, 20.1; ^{13}C-coupled HSQC, see Table 2; HRESIMS m/z 199.1804 [M + H]$^+$(calcd for C$_{11}$H$_{23}$N$_2$O, 199.1805).

(*S*)-5-Isobutyl-1-methyl-3-hydroxymethylimidazolidin-4-one (7). To a stirred solution of (*S*)-N-Boc-N-methylleucine (81 mg, 0.33 mmol) and N-methylmorpholine (7 μL, 0.33 mmol) in dry THF was added isobutyl chloroformate (43 μL, 0.33 mmol) at −15 °C. After the reaction mixture was stirred for 20 min at −15 °C, NH$_3$ gas was bubbled into the reaction mixture for 5 min at −15 °C and then for an additional 1 h before diluting with H$_2$O and extraction with EtOAc (×3). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated under reduced pressure, and the residue was purified by flash chromatography (SiO$_2$, 1:2 EtOAc/hexane) to give the corresponding leucinamide (62 mg, 77%), which was used to prepare (*S*)-7 as described above. (*S*)-7: $[\alpha]_D$ +29 (c 0.01, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 4.89 (d, 1H, J = 10.9 Hz), 4.73 (d, 1H, J = 10.9 Hz), 4.49 (d, 1H, J = 5.3 Hz), 3.94 (dd, 1H, J = 5.3, 1.5 Hz), 2.90 (m, 1H), 2.45 (s, 3H), 1.90 (m, 1H), 1.61 (m, 1H), 1.44 (m, 1H), 0.96 (d, 3H, J = 6.4 Hz), 0.93 (d, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.7, 69.0, 65.4, 65.3, 40.6, 39.1, 24.9, 22.9, 22.8; ^{13}C-coupled HSQC, see Table 2; HRESIMS m/z 187.1441 [M + H]$^+$ (calcd for C$_9$H$_{19}$N$_2$O$_2$ 187.1447).

Molecular Modeling. MMFF calculations of 6 were conducted with Spartan 08 (Wavefunction) using the MM-minimized conformation as a starting point. The 25 lowest energy conformations were found (Monte Carlo), and the relative energies and Boltzmann weightings (%) of the lowest four were calculated to be −1.88 kcal mol^{-1} (36.4%), −0.56 (21.3), 1.47 (9.4), and 2.42 (6.4). See Figure 2.

Cytotoxicity Assays. Cytotoxicity was measured with cultured HCT-116 cells (ATCC CCL-247) by measuring growth inhibition in the presence of compound in 96-well microtiter plates. Cells (density = 1.2 × 10^4 cells/mL) in McCoy's 5A Media containing 10% FBS (175 mL of cell suspension per well) were incubated at 37 °C under 5% CO$_2$ for 24 h prior to addition of extract or compound (in triplicate). IC$_{50}$ was measured from treatments using 8 points in a dilution series from 125 to 0.00763 mg/mL or 125 to 0.00763 ng/mL. Cells were incubated with solutions of extract or pure compound in DMSO (0.89% v/v) for 72 h (3 days) prior to addition of MTS reagent (25 mL of 1.9 mg/mL MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) with 0.044 mg/mL PMS (phenazine methosulfate) in PBS) and further incubated for 3 h before measuring the absorbance at λ = 490 nm using a microplate reader (Molecular Probes Datamax Pro) and analyzed with native instrument

software (SoftmaxPro). The corresponding IC_{50} values for the compounds were measured and reported here as follows: **1**, 32 nM; **2**, 9.0 nM; **4**, 24 nM.

ASSOCIATED CONTENT

ⓢ Supporting Information. ^{1}H,^{13}C NMR and 2D NMR spectra of **4**, ^{1}H, ^{13}C spectra of **5**–**8**, and the ^{13}C-coupled HSQC spectrum of **5**. These materials are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel: +1 (858) 534-7115. Fax: +1 (858) 822-0386. E-mail: tmolinski@ucsd.edu.

ACKNOWLEDGMENT

REFERENCES

(1) (a) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857–1859. (b) Sakai, R.; Stroh, J. G.; Sullins, D. W.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 3734–3748. (c) Rinehart, K. L. U.S. Patent 5,294,603, Mar 15, 1994. (d) Rinehart, K. L.; Lithgow-Bertelloni, A. M. . PCT Int. Pat. Appl. WO 91.04985, Apr 18, 1991.

(2) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69–85.

(3) (a) Chun, H. G.; Davies, B.; Hoth, D.; Suffness, M.; Plowman, J.; Flora, K.; Grieshaber, C.; Leyland-Jones, B. *Invest. New Drugs* **1986**, *4*, 279–284. (b) Rinehart, K. *Med. Res. Rev.* **2003**, *1*, 1–27. (c) Jimeno, J.; Lopez-Martin, J. A.; Ruiz-Casado, A.; Izquierdo, M. A.; Scheuer, P. J.; Rinehart, K. *Anticancer Drugs* **2004**, *15*, 321–329.

(4) Vera, M. D.; Joullie, M. M. *Med. Res. Rev.* **2002**, *22*, 102–145.

(5) (a) Shin, D. M.; Holoye, P. Y.; Murphy, W. K.; Forman, A.; Papasozomenos, S. C.; Hong, W. K.; Raber, M. *Cancer Chemother. Pharmacol.* **1991**, *29*, 145–149. (b) Benvenuto, J. A.; Newman, R. A.; Bignami, G. S.; Raybould, T. J.; Raber, M. N.; Esparza, L.; Walters, R. S. *Invest. New Drugs* **1992**, *10*, 113–117. (c) Kucuk, O.; Young, M. L.; Habermann, T. M.; Wolf, B. C.; Jimeno, J.; Cassileth, P. A. *Am. J. Clin. Oncol.* **2000**, *23*, 273–277.

(6) Lievens, S. C. . Ph.D. Thesis, University of California, Davis, 2006, pp 55–60.

(7) McKee, T. C.; Ireland, C. M.; Lindquist, N.; Fenical, W. *Tetrahedron Lett.* **1989**, *30*, 3053–3056.

(8) The conditions of isolation of **4** likely render the imidazolidinone ring as the HCl salt; however, **5**–**8** were isolated as their free bases.

(9) Of the two unusual long-range ^4J scalar homonuclear couplings between CH_2-64 and H-58 detected by COSY, only one was resolved in the 1D ^{1}H NMR. ^4J(H64α-H58) = 1.2 Hz. See also ref 15.

(10) Gloer, J. B., Ph.D. Thesis, . University of Illinois, Urbana, 1983.

(11) Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1989**, *54*, 4256–4259.

(12) (a) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, 1037–1040. (b) Pretsch, E.; Bühlmann, P.; Badertscher, M. *Structure Determination of Organic Compounds. Tables of Spectra Data*, 4th ed.; Springer: Heidelberg, 2009; p 149.

(13) Lee, G. M.; Molinski, T. F. *Tetrahedron Lett.* **1992**, *33*, 7671–7674.

(14) (a) von Pfanz, H.; Kirchner, G. *Liebigs Ann. Chem.* **1958**, *614*, 149–158. (b) Gesson, J. P.; Jacquesy, J. C.; Mondon, M. *Synlett* **1990**, 669–670.

(15) Strictly, the ^{1}H NMR spectrum of **4** is ABX, where the X proton, the α-proton of the *N*-methyleucyl residue (H-58), shows weak long-range homonuclear coupling (^4J = 1.2 Hz) to only one of the *N*-CH_2-*N* signals: H64α but not H64β (the stereoassignments of these protons have not been made). See Table 1. Collectively, synthetic imidazolidinones **5**–**8** show variable ^{1}H spin patterns from AB in **8**, with zero long-range homonuclear couplings, to ABX in **5**–**7** with $^2J_{AB} \approx -5.3$ Hz, $^4J_{AX} \approx 1.5$–1.7 Hz, and $^4J_{BX} \approx 0$ Hz.

(16) Carbinolamines that are formally derived as adducts of peptides with formaldehyde would be expected to show pseudomolecular ions in their MS spectra that are higher by 30 amu mass units, but—as we observed for **7**—their lability under ESIMS ionization conditions is misleading. The reactive formaldehyde equivalent is easily lost, probably in the heated capillary of the ionization stage, and the expected pseudomolecular ions are absent or replaced by different product ions.

(17) (a) Molinski, T. F. *Nat. Prod. Rep.* **2010**, *27*, 321–329. (b) Dalisay, D. S.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 739–744. (c) Molinski, T. F. *Curr. Opin. Biotechnol.* **2010**, *21*, 819–826.

(18) We cannot rule out the possibility that **4** is the product of adventitious condensation of didemnin A (**1**) with formaldehyde present as a contaminant in the MeOH solvent used during isolation–purification, although this seems unlikely, as the technical specifications of the HPLC grade MeOH state "<0.001% formaldehyde". LC-MS analysis (C_{18} reversed phase) of the whole MeOH extract of *T. solidum* showed the presence of a peak that could be assigned to **4** (m/z 955 [M + H]$^+$, 977 [M + Na]$^+$).