Total Structures of Cryptophycins, Potent Antitumor Depsipeptides from the Blue-Green Alga *Nostoc* sp. Strain GSV 224†

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Abstract: Cryptophycin (A, **1**), the major cytotoxin in the blue-green alga (cyanobacterium) *Nostoc* sp. GSV 224, shows excellent activity against solid tumors implanted in mice. This cyclic depsipeptide had previously been isolated from *Nostoc* sp. ATCC 53789 as an antifungal agent and its gross structure determined by researchers at Merck. The relative and absolute stereochemistry of this potentially important drug has now been established using a combination of chemical and spectral techniques. Six minor cryptophycins (B–G, **2–7**) have also been isolated from GSV 224 and their total structures and cytotoxicities determined. Two types of cryptophycins are present in this alga, the major series possessing a monochlorinated L-*O*-methyltyrosine unit and the minor series possessing a nonchlorinated D-*O*-methyltyrosine unit. Structure–activity relationship (SAR) studies of the cryptophycins and several derivatives and degradation products (**8–14**) are described. Also presented are preliminary *in vivo* results for cryptophycin A against six solid tumors.

Introduction and Discussion

In screening extracts of over 1000 blue-green algae (cyanobacteria) for antitumor activity, we found the lipophilic extract of *Nostoc* sp. GSV 224 to be strongly cytotoxic,[1] exhibiting MICs of 0.24 ng/mL against KB, a human nasopharyngeal carcinoma cell line, and 6 ng/mL against LoVo, a human colorectal adenocarcinoma cell line. More importantly, this extract showed significant tumor-selective cytotoxicity in the Corbett assay.[2] Bioassay-monitored reversed-phase chromatography of the algal extract led to a fraction which was predominantly cryptophycin (A, **1**, Chart 1),[3] a potent fungicide that had been isolated earlier from *Nostoc* sp. ATCC 53789 by researchers at Merck[4,5] and found to be very active against strains of *Cryptococcus*. A gross structure was proposed for **1**, but no details were presented. No further studies were carried out by the Merck group, since **1** was found to be too toxic for use as an antifungal agent.[6]

Cryptophycin A accounted for most of the cytotoxic activity of the crude algal extract of *Nostoc* sp. GSV 224, and the pure compound showed IC_{50} values of 3 and 5 pg/mL against KB and LoVo, respectively. In the Corbett assay, **1** was found to be strongly tumor selective and equally cytotoxic against drug-sensitive and drug-resistant tumor cells. It appeared to have the same mode of action as vinblastine but differed from the latter drug in irreversibly inhibiting tubulin polymerization into microtubules.[7] In preliminary *in vivo* experiments, **1** exhibited excellent activity against tumors implanted in mice.

Minor amounts of several other cryptophycins were present in *Nostoc* sp. GSV 224, and six of these could be isolated in sufficient quantities by reversed-phase HPLC for structure determination and antitumor evaluation *in vitro*. Cryptophycins B (**2**), C (**3**), D (**4**), E methyl ester (**5**), and F methyl ester (**6**), all shown in Chart 1, accompanied **1** in the fraction eluted from a reversed-phase flash column with 3:1 methanol/water. Cryptophycin G (**7**, Chart 1) was found in an earlier, less cytotoxic fraction eluted with 1:3 methanol/water. The gross structures of **3** and **5** appeared to be related to those of fungicidal semisynthetic compounds prepared from **1** by researchers at Merck.[8] We describe here details on the total structure elucidation of **1–7** and preliminary results of structure–activity relationship studies and primary evaluation of these potentially useful antitumor drugs.

Total Structure Determination. Confirmation of the gross structure of cryptophycin A (**1**) was carried out in a straightforward manner. Mass spectral data were consistent with the molecular composition $C_{35}H_{43}ClN_2O_8$. The proton and carbon NMR data obtained from COSY, HMQC, HMBC, and NOESY spectra revealed the presence of units from two hydroxy acids, viz. 7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2-octenoic acid (unit A, R_1, R_2 = O) and 2-hydroxy-4-methylvaleric acid (unit D, leucic acid), and two amino acids, viz. 3-(3-chloro-4-methoxy-

† This paper is dedicated to the memory of Dr. Gerald Grindey.
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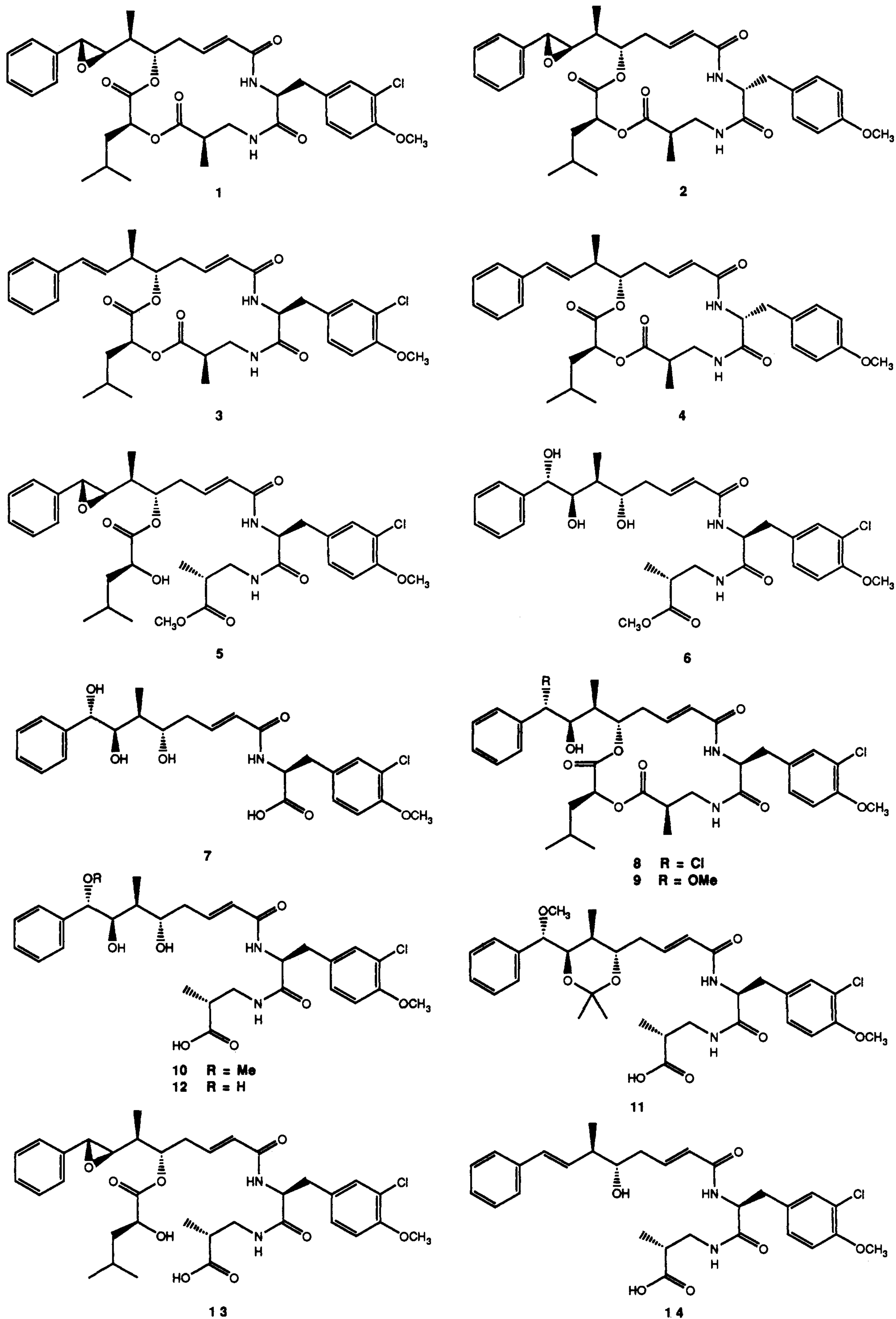
Chart 1



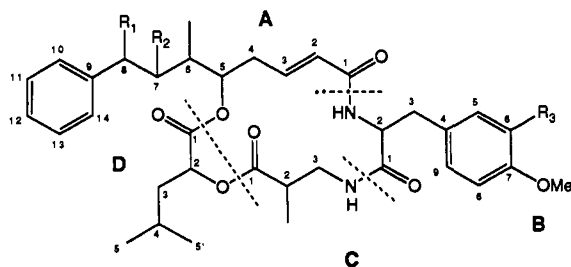


Figure 1. General structure of the cryptophycins and numbering system for the two hydroxy acid units A and D and the two amino acid units B and C. This numbering system is used for the NMR data.

phenyl)alanine (unit B, R_3 = Cl) and 3-amino-2-methylpropionic acid (unit C). These four units could be connected together into a gross structure for **1** (Figure 1) from key HMBC spectral data, viz. $^2J_{H,C}$ and $^3J_{H,C}$ correlations from (1) the unit C NH signal at 6.93 ppm to the unit B carbonyl signal at 170.9 ppm, (2) the unit B NH signal at 5.61 ppm to the unit A carbonyl signal at 165.3 ppm, and (3) the unit A oxymethine proton signal at 5.16 ppm to the unit D carbonyl signal at 170.7 ppm. A correlation peak, however, was not observed between the unit D oxymethine proton signal and the unit C carbonyl signal in the HMBC spectrum since the coupling was too small between these two nuclei ($^3J_{H,C} \leq 3$ Hz); however, a selective INEPT experiment permitted us to see this coupling, thereby providing NMR evidence that units C and D were connected together by an ester linkage.

Chemical shift data supported the attachment of an epoxide oxygen to C-7 (δ_C 63.0, δ_H 2.92) and C-8 (δ_C 59.0, δ_H 3.69) of unit A. Chemical reactivity provided further evidence for the presence of an epoxide functionality since **1** could be readily converted to a chlorohydrin (**8**) by HCl in 2:1 dimethoxyethane/water and to a diol monomethyl ether (**9**) by dilute methanolic HCl. The geometries of the double bond and the epoxide ring in unit A were concluded to be both *trans* from the sizes of the coupling constants $J_{2,3}$ (15.5 Hz) and $J_{7,8}$ (2.0 Hz). As expected, **1** could be regenerated by treating **8** with potassium carbonate in dry acetone.

Determination of the absolute configurations of the four units in **1** required a combination of chemical degradation and spectral analysis. First **1** was subjected to vigorous acid hydrolysis. Derivatization of the amino acids in the acid hydrolysate with Marfey's reagent[9] and comparison of their HPLC retention times with those of standards prepared from authentic D-(*R*)- and L-(*S*)-3-(3-chloro-4-methoxyphenyl)alanine[10] and D-(*R*)- and L-(*S*)-3-amino-2-methylpropionic acid[11] (AMPA) permitted us to assign the L-(*S*) configuration to unit B in **1**. No decision, however, could be made on the absolute stereochemistry of unit C. In our hands, the Marfey derivatives of the (*R*)- and (*S*)-AMPA had identical retention times. The absolute configuration of unit C was solved by GC–MS analysis of the *N*-(pentafluoropropionyl) isopropyl ester derivative on a chiral column.[12] The derivatized AMPA from **1** had a retention time (t_R) that was identical with that of the D-(*R*)-AMPA standard and shorter than that of the corresponding L-(*S*) derivative. The absolute stereochemistry of the leucic acid from **1** was deduced to be L since it was levorotatory[13] and had an R_f value from chiral TLC analysis that was identical with that of the authentic L-enantiomer.[14]

To elucidate the relative and absolute stereochemistry of unit A, **1** was treated with methanolic hydrochloric acid, and the resulting **9** was saponified to a diol (**10**), which was subsequently converted into an acetonide (**11**). The gross structure of **11** was readily confirmed from COSY and HMBC spectral data. The chemical shifts of the acetonide ketal (102.8 ppm) and methyl carbons (23.8 and 24.4 ppm)[15] and the coupling constants between the methine protons in the acetonide ring[16] of **11** suggested that an acetonide of an *anti*-1,3-diol had been produced. The C-6 methine proton signal at 1.97 ppm showed a 7.9-Hz coupling to the C-5 oxymethine proton signal at 3.40 ppm and a 4.9-Hz coupling to the C-7 oxymethine proton at 3.85 ppm, essentially identical with the coupling constants observed in *trans*-4,6-dialkyl-2,2,5-trimethyl-1,3-dioxanes.[16] The magnitude of these values, which reflected the twist-boat conformation for this acetonide (Figure 2), indicated that H-5 and H-6 were *anti* to each other and that H-7 was *syn* to H-6.

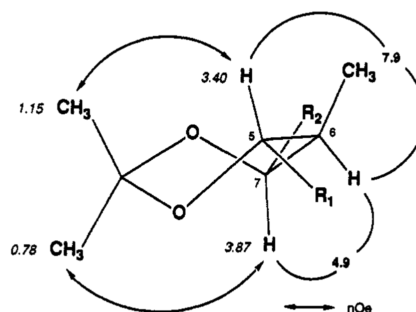


Figure 2. Coupling constant and NOE data providing evidence for relative stereochemistry and twist-boat conformation of the acetonide ring in **11**.

One- and two-dimensional NOE studies of **11** confirmed the relative stereochemistry. As expected for a twist-boat conformation (Figure 2), the H-5 signal at 3.40 ppm exhibited strong NOEs to the acetonide methyl (*syn* to H-5) signal at 1.15 ppm and the H-8 signal at 4.00 ppm, whereas the H-7 signal at 3.85 ppm displayed a strong NOE to the acetonide methyl (syn to H-7) signal at 0.78 ppm. The protons of the equatorial methyl group on C-6 (1.00 ppm) also showed a strong NOE to H-8 and to the methoxyl signal (3.12 ppm) as well. The protons on C-4 showed a strong NOE to the axial C-6 proton, which was consistent with C-4 being connected equatorially to the acetonide. Finally, the *trans* geometry for the epoxide ring in unit A was further supported by NOEs between H-7 and H-10/14 and between H-8 and H-10/14. If the geometry had been *cis*, a NOE would not have been observed between H-7 and H-10/14. We could now conclude that the relative stereochemistry of unit A in **1** was 5*S**,6*S**,7*R**,8*R**.

The determination of the absolute stereochemistry of unit A in **1** was achieved by the modified Mosher method.[17] The *O*-(*R*)- and -(*S*)-MTPA (2-methoxy-2-(trifluoromethyl)-2-phenylacetyl) derivatives of **9** were prepared, and $\Delta\delta$ values ($\delta_S - \delta_R$) were determined at 500 MHz for all of the assignable protons on unit A. Positive $\Delta\delta$ values were found for all of the protons on the C1–C6 side of the MTPA plane, whereas negative values were found for protons on the C8–C14 side (Figure 3). This meant that C-7 had to have the *R* configuration and furthermore that the absolute stereochemistry of unit A had to be 5*S*,6*S*,7*R*,8*R*, as shown in **1**.

Cryptophycin B (**2**) was found to have the molecular formula $C_{35}H_{44}N_2O_8$ from mass and NMR spectral data. Analysis of the proton and carbon NMR data with the aid of COSY and HMQC spectra indicated that **2** had a gross structure that was similar to that of **1**, except that unit B was an *O*-methyltyrosine unit

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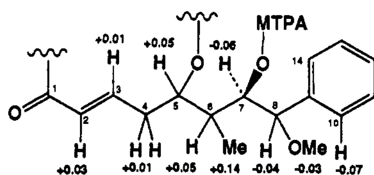


Figure 3. $\Delta\delta$ ($\delta_S - \Delta_R$) values in ppm obtained at 500 MHz for the MTPA esters of **9**.

(Figure 1, R_3 = H). A typical A_2X_2-type spectrum (δ_A 6.81, δ_X 7.10, J_{AX} = 8.6 Hz) could be seen for the four aromatic protons of this unit as shown by correlations between the two proton signal at 6.81 ppm and the methoxyl signal at 3.78 ppm in the NOESY spectrum and between the two proton signals at 6.81 and 7.10 ppm in the COSY spectrum. The chemical shifts and coupling constants for the protons on C1–C8 of the unit A segment of cryptophycins B and A were virtually identical, and this strongly suggested that unit A had the same absolute stereochemistry in the two compounds. At first it appeared that **2** might simply be dechlorocryptophycin A and differ only in the absence of chlorine. To our complete surprise, Marfey analysis of the acid hydrolysate of **2** indicated that the *O*-methyltyrosine unit had the D-(*R*) configuration, opposite to the L-(*S*) configuration found for unit B in **1**. Further analysis, however, showed that the absolute configurations of the AMPA and leucic acid residues were the same. In conclusion, **2** differed from **1** not only in the absence of chlorine from the *O*-methyltyrosine unit but also in the absolute configuration of this unit.

Cryptophycin C (**3**) had the molecular formula $C_{35}H_{43}ClN_2O_7$ based on its high- resolution mass spectrum. Inspection of the NMR spectral data (proton, carbon, COSY, and HMQC) suggested that **3** possessed essentially the same gross structure as **1**. Epoxide signals, however, were absent in the proton NMR spectrum of **3**. Instead, two olefinic-type proton signals could be found at 6.01 and 6.41 ppm with a coupling of 15.8 Hz between them, assignable to a *trans* double bond. The COSY spectrum established that the double bond was between C7 and C8 in unit A, the same position that the epoxide group occupies in cryptophycin A. The absolute stereochemistries of **3** and cryptophycin A were identical since **3** could be converted to **1** with *m*-chloroperbenzoic acid. Cryptophycin C appeared to be identical with a previously reported semisynthetic compound[8a] prepared by treating **1** with a zinc–copper couple or diphosphorus tetraiodide.

Cryptophycin D (**4**), $C_{35}H_{44}N_2O_7$, has the same structural relationship to cryptophycin C (**3**) as **2** has to **1**. The NMR data clearly showed the presence of an *O*-methyltyrosine unit, and repeated Marfey analysis of the acid hydrolysate indicated that this unit had the D(*R*) configuration. Epoxide signals were also missing in the ^{1}H and ^{13}C NMR spectra of **4**, and instead the olefinic signals that were observed in the ^{1}H and ^{13}C NMR spectra of **3** were present. The A units in **3** and **4** were identical since the chemical shifts and coupling constants for the various protons and carbons were the same for the two compounds. In addition, both compounds displayed similar EI mass spectral fragmentation patterns, as shown in Scheme 1.

Cryptophycin E methyl ester (**5**) was shown to have the elemental composition $C_{36}H_{47}ClN_2O_9$ by mass spectrometry. It differed from the formula for cryptophycin A by CH_4O (32 mass units), the elements of methanol. The presence of a second methoxy signal at 3.64 ppm, a chemical shift typical of a methyl ester, and an exchangeable proton signal at 2.86 ppm in the proton spectrum accounted for the additional CH_4O. The COSY and HMBC spectra allowed us to place the methoxy group at C1 of the AMPA unit and the exchangeable proton on the oxygen attached to C2 of the leucic acid unit, indicating that the ester linkage between the AMPA and leucic acid residues was missing. Treatment of cryptophycin E methyl ester or cryptophycin A with dilute aqueous NaOH yielded the same degradation products, viz. cryptophycin F (**12**) and L-leucic acid, indicating that **5** and **1** had the same absolute stereochemistry.

Methyl ester **5** appears to be an artifact. Compound **5**, which was not isolated when methanol was eliminated from the isolation procedure, was probably being formed by either methylation of naturally occurring cryptophycin E (**13**) or methanolysis of **1** during the isolation. To date, however, we have not been able to isolate **13** in the alga. Sesin and Liesch reported[8b] that **5** or **13** was formed when **1** was allowed to stand at room temperature in anhydrous methanol or 30% aqueous methanol, respectively, and that hydrolysis of **1** to **13** occurs with 1% potassium hydroxide in methanol, 0.1 M sodium hydroxide, aqueous ethanol, aqueous 2-propanol, and other unspecified mildly basic reagents.[8b] In our hands, however, **1** was completely stable in methanol and could be recovered unchanged after standing in this solvent for over a month at room temperature. Moreover, we found that treatment of **1** with 0.03 N sodium hydroxide in 4:1 acetone/water led to **12** rather than **13**. A similar treatment of **3** with 0.01 N NaOH gave **14**. The yield of **5** increased, however, with concomitant decrease of **1** when the time period for the isolation, and hence exposure time to methanol, increased.

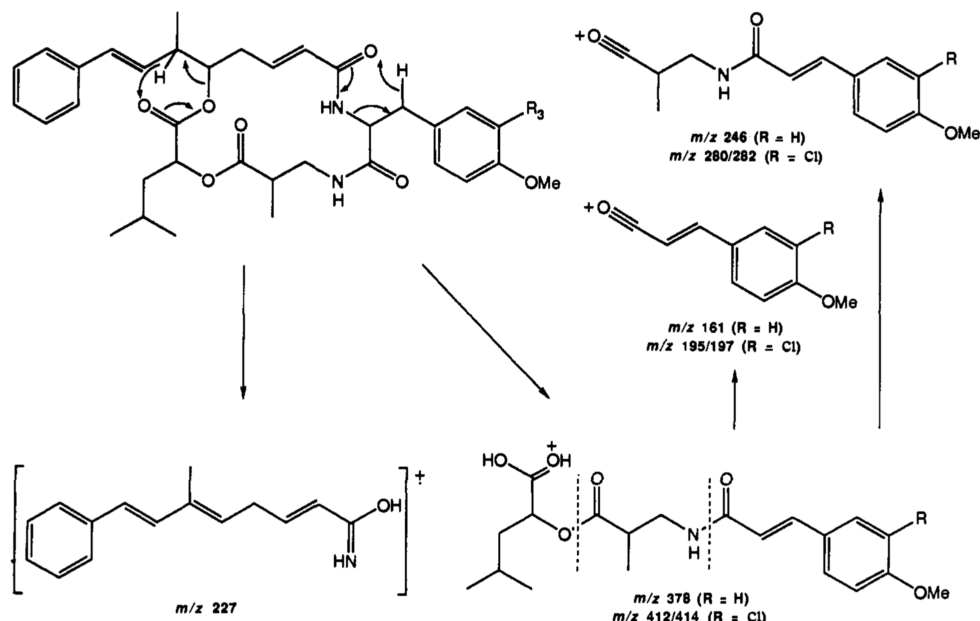
Cryptophycin F methyl ester (**6**), $C_{30}H_{39}ClN_2O_8$, did not exhibit a molecular ion in the EI mass spectrum or an MH^+ ion in the FAB mass spectrum, at least in our hands. A prominent 3:1 ion cluster, however, was observed at *m/z* 573/575 for loss of water from MH^+. Inspection of the ^{1}H and ^{13}C NMR spectra showed proton signals at 3.70, 3.97, and 4.64 ppm and carbon signals at 85.1, 82.5, and 84.8 ppm, respectively, in lieu of the signals for the epoxide group and leucic acid unit. These data suggested that OH groups were on C-5, C-7, and C-8 of unit A. The HMBC spectrum confirmed the assignments and furthermore showed that a methoxyl group was connected to the carbonyl carbon of the AMPA unit. The gross structure of **6** was subsequently proven and the stereochemistry rigorously established by semisynthesis. Cryptophycin A was degraded to a mixture of cryptophycin F (**12**) and L-leucic acid by treating the cytotoxin with dilute aqueous sodium hydroxide. Methylation of **12** with diazomethane produced **6**.

Cryptophycin G (**7**) had to have the molecular formula $C_{25}H_{30}ClNO_7$ based on the NMR data, even though this trihydroxy-carboxylic acid did not show a 3:1 molecular ion cluster at *m/z* 491/493 in the EI mass spectrum or a comparable MH^+ or MNa^+ ion cluster in the FAB mass spectrum. Fragment ions were observed for loss of one and two water molecules from the molecular ion or MH^+ ion. The gross structure elucidation was straightforward from detailed analysis of the proton, carbon and COSY spectral data. Unit B was assigned the L configuration by Marfey analysis of the acid hydrolysate, and Unit A appeared to have the same stereochemistry as in cryptophycin F (**12**) since the proton NMR data for this segment were virtually identical for the two compounds.

Structure–Activity Relationships. The availability of several minor components and semisynthetic analogs possessing a wide range of structural variability provided us with an opportunity to carry out a SAR study and determine some of the structural features in **1** that are responsible for activity.

We first evaluated most of the compounds described in this paper for cytotoxicity against KB and LoVo. KB and LoVo IC_{50} values for **1–10** and **12** are listed in Table 1. The cytotoxicities of epoxides **1** and **2** were two of the strongest and were surprisingly identical in potency, implying that the chloro substituent on the *O*-methyltyrosine and a fixed absolute configuration for this unit were unnecessary for the potent cytotoxicity. Potent cytotoxicity was retained even when **1** was opened by HCl to chlorohydrin **8** but was diminished 1000-fold when the epoxide ring of **1** was opened by methanolysis to **9**. Removal of the epoxide oxygen or hydroxy groups from C-7 and C-8 of unit A as in **3** and **4** resulted in a 100-fold decrease in cytotoxicity. The leucic acid unit was clearly required for the potent activity, since **6**, **7**, **10**, and **12** were only weakly cytotoxic. The ester bond connecting units C and

Scheme 1. Electron Impact Mass Spectral Fragmentation of Cryptophycins C and D[a]



[a] Cryptophycins A and B show similar fragment ions, suggesting that the epoxide oxygen is lost by reduction during the volatilization process on the EI probe.

Table 1. Cytotoxicity Data for Cryptophycins and Semisynthetic Analogs (Corbett/Valeriote Assay Data for 5-Fluorouracil, Etoposide (VP-16), and Taxol Included for Comparison)

	Corbett assay[a]		Valeriote assay[b]			
compd	sample size (μg/disk)	type of cytotoxicity (differential in zone units)	sample size (μg/disk)	type of cytotoxicity (differential in zone units)	KB IC_{50} (ng/mL)	LoVo IC_{50} (ng/mL)
1	12.5	E/T (>400)[c]		N	0.005	0.003
2	25	E/T (>400)[c]		N	0.007	0.0002
3	25	E/T (>400)[c]		N	0.3	0.5
4	20	E/T (>400)[c]		N	1.3	0.5
5	2.9	E/T (>600)[c]		N	2	2
6	250	I			≥100	≥100
7	25	L (290), T (490)[d]			≥750	≥480
8	30	E/T (>500)[c]	30	N	0.0002	0.01
9					15	
10					≥100	≥100
12					≥100	≥100
5-FU	2.5	M/T (>400)[e]	2.5	LL (>400)		
VP-16	5	L (350), T (530)[e]	5	LL (260)		
taxol	0.2	M/H/T (≥400)[e]				

[a] L, leukemia selective (e.g., $Z_{L1210} - Z_{C38}$ and $Z_{L1210} - Z_{H8} \geq 250$ zone units); M, murine solid tumor selective (e.g., $Z_{C38} - Z_{L1210} \geq 250$ zone units); H, human solid tumor selective (e.g. $Z_{H8} - Z_{L1210} \geq 250$ zone units); E, equally cytotoxic toward leukemia and solid tumor cell lines (inhibition zones ≥ 250 zone units); T, tumor selective (e.g., $Z_{L1210} - Z_{LML}$, $Z_{C38} - Z_{LML}$, and $Z_{H8} - Z_{LML} \geq 250$ zone units); I, inactive (inhibition zones < 250 zone units). [b] N, equally cytotoxic toward tumor (leukemia) and normal cell (CFU-GM) lines; LL, lymphocytic leukemia selective ($Z_{L1210} - Z_{CFU\text{-}GM} \geq 250$ zone units); ML, acute myelogenous leukemia (AML) selective ($Z_{AML} - Z_{CFU\text{-}GM} \geq 250$ zone units). [c] $Z_{C38} - Z_{LML}$, $Z_{M17} - Z_{LML}$, and $Z_{H8} - Z_{LML}$. [d] Samples may be contaminated with strongly cytotoxic impurities; retesting is planned with purer compounds. [e] Selective against drug-sensitive cell lines only.

D was also clearly necessary for optimal activity. Cryptophycin E methyl ester (**5**) was 1000-fold less cytotoxic than **1** and **2**.

Next each of the compounds was evaluated for selective cytotoxicity against five different cell types, viz. a murine leukemia (L1210 or P388), a murine solid tumor (colon adenocarcinoma 38, pancreatic ductal adenocarcinoma 03, mammary adenocarcinoma M16), a drug-resistant murine solid tumor (mammary adenocarcinoma M17), a human solid tumor (colon CX-1, HCT8, H116; lung H125; mammary MX-1, MCF-7), and a low-malignacy fibroblast (LML), using the Corbett assay,[2] a disk diffusion assay modeled after the one commonly used in antifungal and antibacterial testing. The results, shown in Table 1, indicated that **1**–**5** and **8** were neither solid tumor nor leukemia selective but rather were equally active against the four tumor cell lines, including the adriamycin-resistant M17. None of the compounds showed a zone of inhibition for any of the solid tumor cell lines that was ≥250 zone units, i.e., ≥7.5 mm, larger than the zone of inhibition for the leukemia cell line. Compounds **1**–**5** and **8**, however, displayed markedly larger zones of inhibition (≥400 zone units larger) for all of the tumor cell lines compared with the zone of inhibition for the fibroblast LML. Diagnostically, LML has been found to behave more like a normal cell than a tumor cell with respect to clinically useful cytotoxic agents (see Corbett assay data for 5-fluorouracil, etoposide, and taxol in Table 1). Since the differential cytotoxicities were >250 zone units, **1**–**5** and **8** were concluded to be tumor selective. These compounds therefore became candidates for *in vivo* testing.

Since experience has shown that the limiting toxicity for an anticancer agent is often myelosuppression, a secondary assay which compares cytotoxicities against leukemias and CFU-GM (a normal myeloid committed stem cell) should select for the most efficacious candidates. In the Valeriote assay,[18] however, which compares cytotoxicity against leukemia cell lines (L1210 lymphocytic leukemia and C1498 acute myelogenous leukemia) with cytotoxicity against CFU-GM, **1**–**5** and **8** were nonselective.

(18) Valeriote, F.; Corbett, T.; Edelstein, M.; Baker, L. *Cancer Inv.*, in press.

Table 2. Cryptophycin A (**1**) Treatment of Mice Bearing Subcutaneous Transplanted Solid Tumors of Mouse and Human Origin

SC tumor, host	IV drug schedule	total dosage (mg/kg)	mean body Wt change (g/mouse)	day of nadir	drug deaths	median tumor burden (mg)		day tumors measured	T/C (%)	tumor-free survivors (day)[a]	log tumor cell kill (T − C)[b]
						control	treated				
colon 38, BDF$_1$ mice	days 3–9[c]	10.3	+0.4	8	0/5	1348	80	14	6	0/5 (33)	1.5[e] (10.7)
colon 51, CDF$_1$ mice	days 1,3,5, 7,8,9,11	28	−2.8	13	0/5	1033	75	17	7	0/5 (22)	1.3[e] (12.5)
pancreatic O3, BDF$_1$ mice	days 3,5,7,9, 11,13,15,17	16	+0.8	19	0/5	1927	0	21	0	0/5 (54)	2.0[e] (18)
DMS273,[d] SCID mice	days 1,3,5, 7,9,11	7.3	0	6	0/5	735	196	12	27	0/5	
mammary 16/C, taxol-sensitive C$_3$H mice	days 1,3,5, 7,9,11	13.2	−0.2	6	0/5	2743	144	12	5	0/5 (21)	1.4[e] (6.5)
mammary 16/C, taxol-resistant C$_3$H mice	days 1,3,5, 7,9	16.5	−0.7	10	0/4	1629	38	11	2	0/4 (18)	1.8[e] (7)

[a] Day on which none of the surviving mice are tumor-free. [b] T is median time (in days) required for the treatment group tumors to reach 750 mg (1000 mg in case of Panc 03); C is median time (in days) for the control group tumors to reach 750 or 1000 mg. Log_{10} tumor cell kill (gross) = $T - C/(3.2)(T_d)$, where T_d is the tumor volume doubling time.[1a] [c] Two times per day on day 4. [d] Human small-cell lung tumor. [e] Gross log kills of >2.8, 2.0–2.8, 1.3–1.9, 0.5–0.8, and <0.5 with duration of drug treatment of 5–20 days are scored ++++, +++, +, and – (inactive), respectively. An activity rating of +++ to ++++ is needed to effect partial or complete regression of 100–300-mg size masses of most transplanted solid tumors of mice.[1a]

To date, only **1** has been evaluated *in vivo*. It is active[19] against all tumors tested so far (Table 2) and exhibits excellent activity against five of them, viz. colon adenocarcinomas 38 and 51, taxol-sensitive and taxol-resistant mammary adenocarcinoma M16, and pancreatic ductal adenocarcinoma 03, showing tumor burden T/C values that are less than 10%.[19] It was also active against lung DMS-273, a highly invasive and highly metastatic human tumor, in the SCID mouse. Lethal toxicity observed during testing was attributed to leucopenia.

Experimental Section

Spectral Analysis. NMR spectra were determined on an 11.75-T instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H NMR chemical shifts are referenced in $CDCl_3$ to residual $CHCl_3$ (7.24 ppm) and in MeOH-d_4 to residual [CH3-d_2] MeOD (3.30 ppm); ^{13}C NMR chemical shifts are referenced in $CDCl_3$ and MeOH-d_4 to the solvent (77.0 and 49.5 ppm, respectively). Homonuclear ^{1}H connectivities were determined with the double-quantum filtered COSY experiment.[20] Homonuclear ^{1}H NOE's were obtained by difference NOE experiments using a 3-s irradiation period and by two-dimensional NOESY and ROESY experiments.[21] One-bond heteronuclear ^{1}H–^{13}C connectivities were determined by proton-detected HMQC[22] and carbon-detected HETCOR experiments. Two- and three-bond ^{1}H–^{13}C connectivities were determined by proton-detected HMBC[23] and carbon-detected selective INEPT experiments.[24] UV and CD spectra and optical rotations were measured in MeOH at 25 °C.

Culture Conditions. *Nostoc* sp. GSV 224 was obtained from Professor C. P. Wolk, MSU-DOE Plant Research Laboratory, Michigan State University. *Nostoc* sp. ATCC 53789 was purchased from the American Type Culture Collection. A 1-L flask culture of alga was used to inoculate an autoclaved 20-L glass carboy containing an inorganic medium, designated BG-11,[1,25] adjusted to pH 7.0 with MOPS. Cultures were continuously illuminated at an incident intensity of 200 μmol photons m^{-2} s^{-1} (photosynthetically active radiation)[26] from banks of cool-white fluorescent tubes and aerated at 5 L/min with 0.5% CO_2 in air at a temperature of 24 ± 1 °C. Typically, cultures were harvested by filtration after 21 days. The yields of lyophilized *Nostoc* sp. GSV 224 and ATCC 53789 averaged 0.61 and 0.3 g/L of culture, respectively.

Isolation. The lyophilized *Nostoc* sp. GSV224 (12.23 g) was extracted twice with 700 and 400-mL portions of MeOH for 12 and 5 h, respectively. The extracts were combined and concentrated *in vacuo* to give 1.84 g of a dark green solid, which was partitioned between water and CH_2Cl_2. The lipophilic portion (0.65 g; KB MIC 0.24 ng/mL) was applied to an ODS-coated silica column (55 g, 7 × 5 cm^2) and subjected to flash chromatography with 1:3 MeOH/H_2O (0.8 L), 1:1 MeOH/H_2O (0.8 L), 3:1 MeOH/H_2O (0.8 L), MeOH (0.8 L), and CH_2Cl_2 (0.5 L). The fraction that was eluted with 3:1 MeOH/H_2O (22 mg; KB MIC 14 pg/mL), which accounted for essentially all of the cytotoxic activity, was subjected to reversed-phase HPLC (Econosil C18, 10 μm, 25 cm × 10 mm, UV detection at 250 nm, flow rate 3 mL/min) using 5:1 MeOH/H_2O as the eluant to give cryptophycins F methyl ester (**6**, t_R 7.6 min, 0.2 mg), E methyl ester (**5**, t_R 15.4 min, 2.3 mg), B (**2**, t_R 16.0 min, 1.0 mg), A (**1**, t_R 19.0 min, 12.0 mg), D (**4**, t_R 26.5 min, 1.2 mg), and C (**3**, t_R 30.2 min, 1.4 mg). Most of methyl ester **5** could be separated from **2** by crystallization from MeOH prior to HPLC. From one of the cultures, the fraction (8.1 mg) that eluted from the flash column with 1:3 MeOH/H_2O showed milder cytoxicity (KB MIC 2 μg/mL). Purification on HPLC (Econosil C18, 10 μm, 25 cm × 10 mm, UV detection at 250 nm) using 3:2 MeOH/H_2O as the eluant yielded cryptophycin G (**7**, t_R 6.0 min, 2.4 mg).

Cryptophycin A (**1**, 2–3 mg) could be isolated from 6 g of lyophilized *Nostoc* sp. ATCC 53789 using the procedure described above.

Analysis of cryptophycins A–F (**1**–**6**) by thin-layer chromatography on C18 silica with 4:1 MeOH/H_2O showed R_f values of 0.30, 0.33, 0.27, 0.28, 0.33, and 0.63, respectively. Cryptophycin G (**7**) showed an R_f value of 0.82 when 3:2 MeOH/H_2O was used as the eluant.

Cryptophycin A (1): $[\alpha]_D$ +33.8° (MeOH, *c* 1.83); UV λ_{max} (ε) 208 (42 400), 218 (33 700), 228 (23 800), 280 (2210); CD $[\theta]_{202}$ +15 900, $[\theta]_{206}$ +64 900, $[\theta]_{214}$ +26 900, $[\theta]_{224}$ +46 300, $[\theta]_{237}$ +10 500. IR ($CHCl_3$) ν_{max} 3425, 2963, 1751, 1719, 1677, 1502, 1259 cm^{-1}; EIMS *m/z* (relative intensity) 654/656 (20/9), 412/414 (33/12), 280/282 (31/12), 227 (80), 195/197 (92/44), 91 (100); high-resolution EIMS *m/z* 654.2665 (calcd for $C_{35}H_{43}ClN_2O_8$, 4.3 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ values (carbon positions, multiplicities; *J* in Hz)] 7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.74 (2, dt; 15.5 and 0.9), 6.68 (3, ddd; 15.5, 9.6, and 5.2), 2.45 (4, ddd; 14.2, 11.1, and 9.6), 2.55 (4, br dd; 14.2 and 5.2), 5.16 (5, ddd; 11.1, 4.9, and 1.9), 1.80 (6, m), 1.14 (6-Me, d; 7.1), 2.92 (7, dd; 7.5 and 2.0), 3.69 (8, d; 2.0), 7.25 (10/14, m), 7.34–7.39 (11/12/13, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.80 (2, ddd; 8.7, 7.3, and 5.4), 5.61 (2-NH, d; 8.7), 3.03 (3, dd; 14.4 and 7.3), 3.13 (3, dd; 14.4, and 5.4), 7.21 (5, d; 2.1), 3.87 (7-OCH_3, s), 6.83 (8, d; 8.5), 7.07 (9, dd; 8.5, and 2.1); 3-amino-2-methylpropionic acid (unit *C*) 2.71 (2, m), 1.22 (2-Me, d; 7.1), 3.30 (3, ddd; 13.4, 5.8, and 3.8), 3.48 (3, ddd; 13.4, 6.3, and 5.8), 6.93 (3-NH, br t; 5.8); leucic acid (unit *D*) 4.83 (2, dd; 6.8, and 3.3), 1.70 (3, m), 1.36 (3, m), 1.70 (4, m), 0.86 (5, d; 6.6), 0.85 (5′, d; 6.6); ^{13}C NMR ($CDCl_3$) [unit δ values (carbon positions)] unit *A* 165.3 (1), 125.3 (2), 141.0 (3), 36.7 (4), 76.2 (5), 40.6 (6), 13.5 (6-Me), 63.0 (7), 59.0 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.5 (12); unit *B* 170.9 (1), 53.6 (2), 35.0 (3), 129.7 (4), 131.0 (5), 122.4 (6), 154.0 (7), 56.1 (7-OCH_3), 112.2 (8), 128.4 (9); unit *C* 175.6 (1), 38.2 (2), 14.1 (2-Me), 41.1 (3); unit *D* 170.7 (1), 71.3 (2), 39.4 (3), 24.5 (4), 22.9 (5), 21.3 (5′).

Cryptophycin B (2): $[\alpha]_D$ +20.4° (MeOH, c 0.54); UV λ_{max} (ε) 206 (43 800), 218 (37 500), 232 (22 900), 278 (2410); CD $[\theta]_{203}$ +54 100,

(19) *T/C* values less than 42% are considered to be active by NCI standards. *T/C* values less than 10% are an indication of excellent antitumor activity by NCI standards.
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(25) Rippka, R.; Deruelles, J.; Waterbury, J. B.; Herdman, M.; Stanier, R. Y. *J. Gen. Microbiol.* **1979**, *111*, 1–61.
(26) Designated μEinstein/m^{2}s in earlier publications.

$[\theta]_{212}$ +16 500, $[\theta]_{225}$ +53 600, $[\theta]_{236}$ −14 000. IR ($CHCl_3$) ν_{max} 3423, 3029, 2961, 1742, 1724, 1678, 1512, 1258 cm^{-1}. EIMS *m/z* (relative intensity, assignment) 620 (11, M^+), 431 (3), 378 (8), 377 (6), 311 (11), 246 (10), 244 (8), 227 (14), 195 (17), 161 (84, CH_3O-C_6H_4—CH=CH=CO^+), 121 (79, CH_3O—C_6H_4—CH_2^+), 91 (100); high-resolution EIMS *m/z* 620.3094 (calcd for $C_{35}H_{44}N_2O_8$, 0.3 mmu error), 161.0605 (calcd for $C_{10}H_9O_2$, −0.2 mmu error), 121.0658 (calcd for C_8H_9O, −0.4 mmu error); ^1H NMR ($CDCl_3$) 7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.71 (2, dd; 15.4, and 1.3), 6.70 (3, ddd; 15.4, 10.2, and 5.0), 2.45 (4, m), 2.55 (4, m), 5.18 (5, ddd; 11.3, 4.8, and 2.0), 1.79 (6, m), 1.14 (6-Me, d; 7.0), 2.92 (7, dd; 7.7, and 2.0), 3.68 (8, d; 2.0), 7.24 (10/14, m), 7.34–7.39 (11/12/13, m); *O*-methyltyrosine (unit *B*) 4.79 (2, ddd; 8.1, 7.0, and 5.7), 5.55 (2-NH, d; 8.1), 3.07 (3, dd; 14.5, and 7.0), 3.13 (3, dd; 14.5, and 5.7), 7.10 (5/9, d; 8.6), 6.81 (6/8, d; 8.6), 3.78 (7-OCH_3, s); 3-amino-2-methylpropionic acid (unit *C*) 2.68 (2, m), 1.23 (2-Me, d; 7.3), 3.39 (3-H_2, m), 7.02 (3-NH, b rt; 6.0); leucic acid (unit *D*) 4.82 (2, dd; 10.1, and 3.7), 1.70 (3, m), 1.33 (3, m), 1.70 (4, m), 0.86 (5, d; 6.4); 0.84 (5′, d; 6.4); ^{13}C NMR ($CDCl_3$): unit *A* 165.1 (1), 125.1 (2), 141.1 (3), 36.7 (4), 76.0 (5), 40.7 (6), 13.6 (6-Me), 63.0 (7), 59.0 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.5 (12); unit *B* 171.1 (1), 53.9 (2), 35.3 (3), 131.0 (4), 130.2 (5/9), 114.1 (6/8), 158.6 (7), 55.2 (7-OCH_3); unit *C* 176.0 (1), 38.1 (2), 14.2 (2-Me), 40.7 (3); unit *D* 170.6 (1), 71.3 (2), 39.4 (3), 24.5 (4), 21.3 (5), 22.9 (5′).

Cryptophycin C (3): $[\alpha]_D$ +20.3° (MeOH, c 1.13); UV λ_{max} (ϵ) 206 (51 700), 218 (31 200), 230 (22 900), 246 (18 800), 280 (3230); CD $[\theta]_{205}$ +50 000, $[\theta]_{212}$ −90, $[\theta]_{218}$ −47 200, $[\theta]_{233}$ −100, $[\theta]_{251}$ +33 400, $[\theta]_{271}$ +4310. IR ($CHCl_3$) ν_{max} 3417, 2926, 1742, 1721, 1676, 1499, 1336 cm^{-1}; EIMS *m/z* (relative intensity) 638/640 (2/0.7, M^+), 412/414 (63/19), 280/282 (15/5), 227 (100), 195 (63), 91 (98); high-resolution EIMS *m/z* 638.2764 (calcd for $C_{35}H_{43}ClN_2O_7$, −0.5 mmu error), 412.1516 (calcd for $C_{20}H_{27}ClNO_6$, 1.1 mmu error), 227.1293 (calcd for $C_{15}H_{17}$-NO, 1.0 mmu error); ^1H NMR ($CDCl_3$) 5-hydroxy-6-methyl-8-phenyl-2,7-octadienoic acid (unit *A*) 5.77 (2, d; 15.5), 6.68 (3, ddd; 15.5, 9.5, and 5.3), 2.37 (4, m), 2.54 (4, m), 5.01 (5, ddd; 11.4, 6, and 1.5), 2.56 (6, m), 1.14 (6-Me, d; 7.0), 6.01 (7, dd; 15.8, and 8.8), 6.41 (8, d; 15.8), 7.28–7.34 (10/11/13/14, m), 7.23 (12, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.82 (2, m), 5.64 (2-NH, d; 8.8), 3.05 (3, dd; 14.5, and 7.0), 3.13 (3, dd; 14.5, and 5.5), 7.22 (5, d; 2.2), 3.87 (7-OCH_3, s), 6.84 (8, d; 8.5), 7.08 (9, dd; 8.5, and 2.2); 3-amino-2-methylpropionic acid (unit *C*) 2.71 (2, m), 1.22 (2-Me, d; 7.3), 3.28 (3, dt; 13.5, and 7.0), 3.50 (3, ddd; 13.5, 4.9, and 4), 6.93 (3-NH, br t; 6.3); leucic acid (unit *D*) 4.84 (2, dd; 10.1, and 3.6), 1.62 (3, m), 1.36 (3, m), 1.62 (4, m), 0.77 (5, d; 6.5), 0.73 (5′, d; 6.3); ^{13}C NMR ($CDCl_3$) unit *A* 165.4 (1), 125.2 (2), 141.4 (3), 36.5 (4), 77.1 (5), 42.3 (6), 17.3 (6-Me), 130.1 (7), 130.0 (8), 136.7 (9), 126.1 (10/14), 128.6 (11/13), 128.4 (12); unit *B* 170.1 (1), 53.5 (2), 35.1 (3), 129.8 (4), 131.0 (5), 122.4 (6), 154.0 (7), 56.1 (7-OCH_3), 112.2 (8), 127.6 (9); unit *C* 175.6 (1), 38.3 (2), 14.0 (2-Me), 41.2 (3); unit *D* 170.1 (1), 71.6 (2), 39.5 (3), 24.5 (4), 21.2 (5), 22.7 (5′).

Cryptophycin D (4): $[\alpha]_D$ +36.7° (MeOH, c 1.93); UV λ_{max} (ϵ) 206 (41 800), 228 (25 000), 240 (21 200), 248 (22 500), 280 (3000), 290 (1230); CD $[\theta]_{205}$ +63 900, $[\theta]_{211}$ +3040, $[\theta]_{218}$ −71 900, $[\theta]_{229}$ −11 700, $[\theta]_{234}$ −130, $[\theta]_{252}$ +47 500, $[\theta]_{270}$ +5400; IR ($CHCl_3$) ν_{max} 3410, 2962, 2917, 1741, 1718, 1678, 1511, 1251 cm^{-1}; EIMS *m/z* (relative intensity) 604 (2, M^+), 378 (74), 246 (11), 227 (46), 161 (100), 91 (96); high-resolution EIMS *m/z* 604.3127 (calcd for $C_{35}H_{44}N_2O_7$, 2.2 mmu error), 378.1910 (calcd for $C_{20}H_{28}NO_6$, 0.7 mmu error), 227.1293 (calcd for $C_{15}H_{17}NO$, 1.7 mmu error), 161.0605 (calcd for $C_{10}H_9O_2$, −0.2 mmu error); ^1H NMR ($CDCl_3$) 5-hydroxy-6-methyl-8-phenyl-2,7-octadienoic acid (unit *A*) 5.74 (2, dd; 15.3, and 1.2), 6.71 (3, ddd; 15.3, 10.3, and 5.0), 2.37 (4, m), 2.53 (4, m), 5.03 (5, ddd; 11.2, 6.4, and 2.0), 2.55 (6, m), 1.13 (6-Me, d; 6.8), 6.01 (7, dd; 15.8, and 8.8), 6.40 (8, d; 15.8), 7.28–7.37 (10/11/13/14, m), 7.22 (12, m); *O*-methyltyrosine (unit *B*) 4.79 (2, m), 5.61 (2-NH, d; 7.8), 3.08 (3, dd; 14.5, and 7.0), 3.13 (3, dd; 14.5, and 5.3), 7.11 (5/9, d; 8.8), 6.81 (6/8, d; 8.8), 3.78 (7-OCH_3, s); 3-amino-2-methylpropionic acid (unit *C*) 2.69 (2, m), 1.22 (2-Me, d; 7.5), 3.39 (3-H_2, m), 7.03 (3-NH, br t; 6.0); leucic acid (unit *D*) 4.84 (2, dd; 10.1, and 3.6), 1.65 (3, m), 1.34 (3, m), 1.65 (4, m), 0.75 (5, d; 6.5), 0.72 (5′, d; 6.3); ^{13}C NMR ($CDCl_3$) unit *A* 165.3 (1), 125.1 (2), 141.5 (3), 36.5 (4), 77.1 (5), 42.3 (6), 17.3 (6-Me), 130.1 (7), 131.8 (8), 136.7 (9), 126.2 (10/14), 128.7 (11/13), 127.6 (12); unit *B* 171.2 (1), 53.8 (2), 35.3 (3), 131.0 (4), 130.2 (5/9), 114.1 (6/8), 158.6 (7), 55.2 (7-OCH_3); unit *C* 175.9 (1), 38.2 (2), 14.2 (2-Me), 40.9 (3); unit *D* 170.8 (1), 71.6 (2), 39.5 (3), 24.5 (4), 21.2 (5), 22.7 (5′).

Cryptophycin E Methyl Ester (5): $[\alpha]_D$ +36.0° (MeOH, c 0.55); UV λ_{max} (ϵ) 206 (45 600), 218 (37 700), 280 (3790), 286 (3480), 325 (2080); CD $[\theta]_{203}$ +7710, $[\theta]_{206}$ +29 000, $[\theta]_{210}$ +21 400, $[\theta]_{222}$ +59 800, $[\theta]_{234}$ +12 800, $[\theta]_{241}$ +13 700; IR ($CHCl_3$) ν_{max} 3426, 2958, 1728, 1672, 1502, 1259 cm^{-1}; EIMS *m/z* (relative intensity) 686/688 (0.15/0.05), 655/657 (1/0.3), 654/656 (1.5/0.5), 311/313 (75/27), 195 (66), 155 (54), 121 (51), 91 (100); high-resolution EIMS *m/z* 686.2983 (calcd for $C_{36}H_{47}ClN_2O_9$, −1.3 mmu error); ^1H NMR ($CDCl_3$) 7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.87 (2, d; 15.3), 6.72 (3, dt; 15.3, and 6.8), 2.60 (4, m), 2.52 (4, ddd; 15.2, 7.8, and 6.8), 5.11 (5, ddd; 12.3, 7.8, and 7.1), 1.87 (6, m), 1.12 (6-Me, d; 7.1), 2.91 (7, dd; 7.3, and 2.1), 3.70 (8, d; 2.1), 7.24 (10/14, br d; 7.4), 7.29–7.36 (11/12/13, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.59 (2, dt; 6, and 7.5), 6.30 (2-NH, d; 7.5), 2.95 (3, dd; 13.6, and 7.5), 3.0 (3, dd; 13.6, and 6.0), 7.2 (5, d; 2.1), 3.86 (7-OCH_3, s), 6.84 (8, d; 8.5), 7.05 (9, dd, 8.5; 2.1); 3-amino-2-methylpropionic acid (unit *C*) 3.64 (1-OCH_3, s), 2.60 (2, m), 1.07 (2-Me, d; 7.3), 3.27 (3, ddd; 13.5, 8.0, and 5.5), 3.39 (3, m), 6.32 (3-NH, t; 5.4); leucic acid (unit *D*) 4.09 (2, m), 2.86 (2-OH, br d, 6.1), 1.83 (3, m), 1.42 (3, m), 1.86 (4, m), 0.90 (5, d; 6.6), 0.87 (5′, d; 6.8); ^{13}C NMR ($CDCl_3$) unit *A* 164.8 (1), 126.5 (2), 139.2 (3), 34.4 (4), 75.5 (5), 39.2 (6), 12.9 (6-Me), 63.3 (7), 58.7 (8), 136.8 (9), 125.7 (10/14), 128.6 (11/13), 128.4 (12); unit *B* 170.6 (1), 54.6 (2), 37.4 (3), 129.5 (4), 131.0 (5), 122.4 (6), 154.1 (7), 56.1 (7-OMe), 112.2 (8), 128.4 (9); unit *C* 175.4 (1), 51.9 (1-OMe), 39.1 (2), 14.7 (2-Me), 41.6 (3); unit *D* 175.1 (1), 69.2 (2), 43.2 (3), 24.3 (4), 21.2 (5), 23.2 (5′).

Cryptophycin F Methyl Ester (6): $[\alpha]_D$ +17.1° (MeOH, c 1.1); UV λ_{max} (ϵ) 206 (40 000), 218 (30 100), 228 (21 400), 282 (2 430); CD $[\theta]_{203}$ +37 700, $[\theta]_{210}$ −5430, $[\theta]_{213}$ −1260, $[\theta]_{221}$ +24 100, $[\theta]_{232}$ +8480, $[\theta]_{240}$ +13 400, $[\theta]_{254}$ +790; IR ($CHCl_3$) ν_{max} 3425, 3006, 2956, 1726, 1672, 1641, 1502, 1462, 1259 cm^{-1}; FABMS (thioglycerol) *m/z* (relative intensity) 573/575 (13/6) $[M - H_2O]^+$, 217 (26), 91 (100); ^1H NMR ($CDCl_3$) 5,7,8-trihydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.92 (2, dt; 15.0, and 1.5), 6.94 (3, dt; 15, and 7.5), 2.51 (4, m), 2.64 (4, m), 3.97 (5, ddd; 9.3, 6.5, and 4.5), 2.03 (6, m), 1.10 (6-Me, d; 6.5), 3.70 (7, dd; 9.0, and 7.5), 4.64 (8, d; 7.5), 7.33–7.39 (10/11/13/14, m), 7.28 (12, tt; 6.5, and 2.0); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.60 (2, td; 8.0, and 6.0), 6.09 (2-NH, br d; 8.0), 2.96 (3, dd; 13.8, and 8.0), 3.02 (3, dd; 13.8, and 6.0), 7.22 (5, d; 2.0), 3.86 (7-OCH_3, s), 6.84 (8, d; 8.5), 7.07 (9, dd; 8.5, and 2.0); 3-amino-2-methylpropionic acid (unit *C*) 3.63 (1-OCH_3, s), 2.58 (2, m), 1.07 (2-Me, d; 7.0), 3.24 (3, ddd; 13.8, 8, and 6.5), 3.41 (3, ddd; 13.8, 6.5, and 4.8), 6.21 (3-NH, br t; 6.5). ^{13}C NMR ($CDCl_3$) unit *A* 165.2 (1), 125.6 (2), 141.3 (3), 36.9 (4), 82.5 (5), 46.3 (6), 14.3 (6-Me), 85.1 (7), 84.8 (8), 140.9 (9), 125.8 (10/14), 128.6 (11/13), 127.8 (12); unit *B* 170.6 (1), 54.5 (2), 37.3 (3), 129.6 (4), 131.0 (5), 122.5 (6), 154.1 (7), 56.1 (7-OCH_3), 112.2 (8), 128.5 (9); unit *C* 52.0 (1-OCH_3), 175.4 (1), 39.2 (2), 14.7 (2-Me), 41.6 (3).

Cryptophycin G (7): $[\alpha]_D$ −51.9° (MeOH, c 0.89); UV λ_{max} (ϵ) 206 (23 400), 220 (14 900), 282 (1670); CD $[\theta]_{202}$ +35 400, $[\theta]_{206}$ −1730, $[\theta]_{211}$ −19 200, $[\theta]_{220}$ −15 800, $[\theta]_{232}$ +29 000, $[\theta]_{263}$ +2040; IR ($CHCl_3$) ν_{max} 3426, 2946, 1732, 1675, 1501, 1258 cm^{-1}; EIMS *m/z* (relative intensity) 455/457 (1/0.3, $[M - 2H_2O]^+$), 105 (100), 77 (98); FABMS *m/z* (magic bullet matrix) 496/498 $[M - H_2O + Na]^+$, (thioglycerol matrix) 474/476 $[M - H_2O + H]^+$; ^1H NMR (CD_3OD) 5,7,8-trihydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 6.06 (2, ddd; 15.5, 1.3, and 1.0), 6.80 (3, dt; 15.5, and 7.5), 2.49 (4, m), 2.59 (4, m), 3.92 (5, ddd; 9.5, 6.3, and 4.7), 1.95 (6, m), 1.08 (6-Me, d; 6.7), 3.59 (7, dd; 9.0, and 7.8), 4.56 (8, d; 7.8), 7.37 (10/14, br d; 7.3), 7.31 (11/13, br t; 7.3), 7.24 (12, tt; 7.3, and 1.5); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.52 (2, dd; 6.9, and 5.0), 2.93 (3, dd; 13.8, and 6.9), 3.15 (3, dd; 13.8, and 5.0), 7.20 (5, d; 2.2), 3.78 (7-OCH_3, s), 6.88 (8, d; 8.4), 7.08 (9, dd; 8.4, and 2.2); ^{13}C NMR (CD_3OD) unit *A* 167.4 (1), 127.6 (2), 140.9 (3), 37.9 (4), 84.0 (5), 47.6 (6), 14.4 (6-Me), 86.0 (7), 85.8 (8), 142.9 (9), 127.1 (10/14), 129.3 (11/13), 128.5 (12); unit *B* 177.6 (1), 57.3 (2), 38.2 (3), 132.8 (4), 132.1 (5), 122.9 (6), 155.0 (7), 56.5 (7-OCH_3), 113.2 (8), 130.1 (9).

Acid Hydrolysis of the Cryptophycins. (A) Marfey Analysis. Each cryptophycin (1 mg) in 0.5–1 mL of 6 N HCl was heated in a glass tube sealed under vacuum at 110 °C for 12 h. The dried acid hydrolysate in 100 μL of water was treated with 2 mg of N^{α}-(5-fluoro-2,4-dinitro-phenyl)-L-alaninamide (FDAA, Marfey's reagent) in 200 μL of acetone followed by 40 μL of 1 N sodium bicarbonate solution, and the mixture was heated at 40 °C for 1 h. After mixture was cooled to room temperature, 20 μL of 2 N HCl was added, and the resulting solution was analyzed by reversed-phase HPLC on a 250- × 10-mm^2 C18 column (Econosil 10 μm) using a linear gradient of 10–50% MeCN in 0.05 M Et_3N/phosphate buffer at pH 3 over 45 min (isocratic conditions thereafter) at a flow rate of 2 mL/min. Standards were prepared using the same procedure by treating 4 μmol of each authentic amino acid in 80 μL of water with 6 μmol of FDAA in 200 μL of acetone followed by 40 μL of 1 N $NaHCO_3$ solution. The retention times of the FDAA standards were 58.2 min for L-(*S*)-

3-(3-chloro-4-methoxyphenyl)alanine, 63.8 min for D-(*R*)-3-(3-chloro-4-methoxyphenyl)alanine, 56.0 min for L-(*S*)-*O*-methyltyrosine, 58.8 min for D-(*R*)-*O*-methyltyrosine, and 47.6 min for both (*R*)- and (*S*)-3-amino-2-methylpropionic acid. The FDAA-derivatized acid hydrolysates of cryptophycins A, C, E, F, and G showed an HPLC peak at 58.2 min, indicating the presence of an L-(*S*)-3-(3-chloro-4-methoxyphenyl)alanine (CMPHE) unit in each of these chlorine-containing compounds, whereas the FDAA-derivatized acid hydrolysates of cryptophycins B and D showed an HPLC peak at 58.8 min, indicating the presence of an D-(*R*)-*O*-methyltyrosine unit in each of these nonchlorine-containing compounds. Co-injection of derivatized unknown and standard provided further proof.

(B) Chiral GC-MS Analysis. Each cryptophycin (1 mg) was dissolved in 6 N HCl (0.3 mL) and heated at 110 °C for 12 h. The solvent was removed under vacuum, and the dry hydrolysate mixture was treated with a mixture of 0.3 mL of 2-propanol and 0.05 mL of acetyl chloride at 100 °C for 45 min. The excess reagent was then evaporated at 115 °C under a stream of nitrogen, and the residue, which contained the isopropyl esters of the various amino and hydroxy acids in the hydrolysate, was treated with 0.5 mL of 1:1 $(CF_3CF_2CO)_2O/CH_2Cl_2$ at 100 °C for 15 min. After the mixture wasz cooled to ambient temperature, the excess reagent was evaporated with a stream of dry nitrogen, and the resulting mixture of isopropyl esters of *N*-(pentafluoropropyl)amino acids was dissolved in 0.5 mL of CH_2Cl_2 for GC–MS analysis on a 25-m × 0.25-mm Chirasil-Val column (Alltech). The following conditions were used for the GC: a 12-psi head pressure (flow rate estimated to be about 0.6 mL/s) and a column temperature held at 40 °C for 3 min after injection of the sample and then increased from 40 °C to 120 °C at 1.5 deg/min, held at 120 °C for 1 min, and finally increased from 120 °C to 200 °C at 6 deg/min. The same procedure was repeated for standard samples of 3-amino-2-methylpropionic acid (AMPA). The retention times were found to be 25.43 and 25.84 min for the authentic D-(*R*) and L-(*S*) *N*-(pentafluropropionyl) AMPA isopropyl esters, respectively. The retention time for the derivatized AMPA in all of the hydrolysates was 25.43 min.

(C) Chiral TLC Analysis. Each of the acid hydrolysates of cryptophycins A–E described above in parts A and B was subjected to TLC analysis on Chiralplate (Macherey-Nagel) using 1:9 $MeOH/CH_2Cl_2$ as the developing solvent. With a V_2O_5 spray reagent, the leucic acid in the various acid hydrolysates showed intense blue spots having R_f values of 0.65. Authentic L-leucic acid and D-leucic acid showed R_f values of 0.65 and 0.53.

(*R*)- and (*S*)-3-Amino-2-methylpropionic Acid (AMPA). Both compounds were prepared using procedures described in the literature. In one method, the racemic amino acid was converted to a mixture of (*R*)- and (*S*)-*N*-(3-amino-2-methylpropionyl)-L-aspartic acid. The diasteriomers were then separated on a Dowex 50 resin column, and each dipeptide was hydrolyzed to the desired AMPA and L-aspartic acid, which were then separated.[27] In another method, D-(*R*)-3-amino-2-methylpropionic acid was prepared by selectively removing the undesired L-(*S*) enantiomer from the racemic amino acid with *Saccharomyces cerevisiae* (Baker's yeast).[28]

(*R*)- and L-(*S*)-3-(3-Chloro-4-methoxyphenyl)alanine. Commercially available 3-chloro-L-tyrosine (1 mmol) was dissolved in 1 mL of 2 N NaOH (2 mmol), and a solution of 0.125 g (0.5 mmol) of cupric sulfate pentahydrate in 1 mL of water was slowly added. The mixture was heated to 60 °C and then cooled to room temperature. The blue copper complex that formed was redissolved by addition of 0.5 mL (1 mmol) of 2 N NaOH, and the solution was then treated with 284 μL of dimethyl sulfate and vigorously stirred at room temperature for 5 h. The purple-blue precipitate was collected on a filter paper, washed with a mixture of 1:4 $MeOH/H_2O$, and dried. The methylated complex was dissolved in 10 mL of water and acidified to pH 2. The mixture was then aerated with H_2S for 10 min and then with nitrogen for 15 min and filtered. The solvent was removed by lyophilization to give L-(*S*)-3-(3-chloro-4-methoxyphenyl)alanine. Racemic amino acid (actually 3:2 L:D) was obtained by heating a solution of the L-enantiomer with 5 equiv of acetic anhydride in acetic acid at reflux for 30 min.

(*R*)- and L-(*S*)-*O*-Methyltyrosine. According to the procedure described above, D- and L-tyrosine were converted to the corresponding *O*-methyl derivatives.

Converion of 1 to 8. To a solution of 3.8 mg of **1** in 1.5 mL of 2:1 1,2-dimethoxyethane/water was added 9 μL of 1 N HCl. The solution was allowed to stir at room temperature for 4 h, neutralized with potassium carbonate, and evaporated. The residue was partitioned between water and CH_2Cl_2. The CH_2Cl_2-soluble material was purified by reversed-phase HPLC on C_{18} using 4:1 $MeOH/H_2O$ to obtain 3.3 mg of pure **8**: ^1H NMR ($CDCl_3$) 8-chloro-5,7-dihydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.79 (2, d; 15.4), 6.69 (3, ddd; 15.4, 9.7, and 5.5), 2.68 (4, ddt; 14.0, 5.5, and 1.8), 2.38 (4,m), 5.11 (5, ddd; 10.8, 8.6, and 1.8), 2.51 (6, m), 1.05 (6-Me, d; 7.0), 4.01 (7, dd; 9.6 and 1.9), 4.65 (8, d; 9.6), 7.36–7.41 (10/11/12/13/14, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.82 (2, ddd; 8.8, 7.2, and 5.6), 5.64 (2-NH, d; 8.8), 3.03 (3, dd; 15.4 and 7.2), 3.16 (3, dd; 15.4, and 5.6), 7.23 (5, d; 2.2), 3.88 (7-OCH_3, s), 6.85 (8, d; 8.5), 7.09 (9, dd; 8.5 and 2.2); 3-amino-2-methylpropionic acid (unit *C*) 2.73 (2, m), 1.22 (2-Me, d; 7.2), 3.25 (3, ddd; 13.5, 6.8, and 6.1), 3.54 (3, ddd; 13.5, 6.1, and 3.4), 6.91 (3-NH, br t; 6.1); leucic acid (unit *D*) 4.92 (2, dd; 10.1, and 3.5), 1.76 (3/4, m), 1.45 (3, m), 0.94 (5, d; 6.6), 0.94 (5′, d; 6.4).

Conversion of 1 to 9 with Methanolic Hydrochloric Acid. To a solution of 10 mg of **1** in 1 mL of dry methanol was added 10 μL of methanolic HCl (obtained by treating 1.25 g of thionyl chloride with 25 mL of MeOH). After the solution was stirred for 4 h, the solvent was removed in vacuo, and the sample was left under vacuum for 12 h. Reversed-phase HPLC with 4:1 $MeOH/H_2O$ gave 8 mg of pure **9**: ^1H NMR ($CDCl_3$) 5,7-dihydroxy-8-methoxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.76 (2, d; 15.5), 6.67 (3, ddd; 15.5, 9.5, and 5.6), 2.34 (4, ddd; 14.1, 11.1 and 9.5), 2.62 (4, dddd; 14.1, 5.6, 1.8, and 1.5), 5.09 (5, ddd; 11.1, 7.8, and 1.8), 2.24 (6, dqd; 7.8, 7.0, and 2.2), 1.03 (6-Me, d; 7.0), 3.71 (7, dd; 8.3 and 2.2), 4.03 (8, d; 8.3), 3.20 (8-OCH_3, s), 7.31–7.40 (10/11/12/13/14, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.82 (2, ddd; 8.8, 7.4 and 5.6), 5.66 (2-NH, d; 8.8), 3.02 (3, dd; 14.4 and 7.4), 3.15 (3, dd; 14.4 and 5.6), 7.23 (5, d; 2.2), 3.87 (7-OCH_3, s), 6.84 (8, d; 8.5), 7.08 (9, dd; 8.5 and 2.2); 3-amino-2-methylpropionic acid (unit *C*) 2.71 (2, ddq; 6.8, 3.9, and 7.2), 1.21 (2-Me, d; 7.2), 3.23 (3, ddd; 13.5, 6.8, and 6.0), 3.52 (3, ddd; 13.5, 6.0, and 3.9), 6.90 (3-NH, br t; 6.0); leucic acid (unit *D*) 4.86 (2, dd; 9.8 and 3.5), 1.71 (3/4, m), 1.41 (3, m), 0.89 (5/5′, d; 6.4).

Conversion of 8 to 1. A mixture of 2.1 mg of **8** in 1.5 mL of dry acetone was treated with 5 mg of potassium carbonate at 60 °C. After 24 h, the reaction mixture was filtered, the solvent evaporated from the filtrate, and the residue subjected to reversed-phase HPLC on a C_{18} column with 3:1 $MeOH/H_2O$ to give a quantitative yield of **1**.

Saponification of 9. To a stirred solution of 7 mg of **9** in 1 mL of acetone and 0.3 mL of water was added 8 μL of 2 N NaOH. After being stirred for 4 h, the solution was neutralized to pH 7 with 1 N HCl, and the solvent was removed under reduced pressure. The residue was subjected to reversed-phase HPLC (Econosil C_{18}, 10 μm, 25 cm × 10 mm) using 7:3 $MeOH/H_2O$ to yield pure **10** (5 mg): ^1H NMR (CD_3OD) 5,7-dihydroxy-8-methoxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 5.99 (2, dt; 15.4 and 1.3), 6.82 (3, dt; 15.4 and 7.3), 2.30 (4, m), 2.50 (4, m), 3.66 (5, td; 7.8 and 3.5), 2.05 (6, d pentet; 1.9 and 7.0), 0.96 (6-Me, d; 7.0), 4.04 (7, dd; 8.8 and 1.9), 4.01 (8, d; 8.8), 3.12 (8-OCH_3, s), 7.26–7.36 (10/11/12/13/14, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.57 (2, dd; 8.5 and 6.5), 2.82 (3, dd; 13.9 and 8.5), 3.03 (3, dd; 13.9 and 6.5), 7.25 (5, d; 2.2), 3.82 (7-OCH_3, s), 6.96 (8, d; 8.6), 7.13 (9, dd; 8.6 and 2.2); 3-amino-2-methylpropionic acid (unit *C*) 2.50 (2, m), 1.02 (2-Me, d; 7.3), 3.16 (3, dd; 13.4 and 6.9), 3.82 (3, dd; 13.4 and 6.6); ^{13}C NMR (CD_3OD) 179.5, 173.4, 168.2, 155.4, 143.7, 141.7, 131.9, 131.7, 129.8, 129.3 (2), 129.2 (2), 128.8, 126.2, 123.2, 113.4, 85.9, 74.5, 74.1, 56.8, 56.6, 56.3, 43.3, 41.2, 40.2, 38.8, 38.0, 15.5, 9.9.

Acetonide 11. Method I. A mixture of **10** (2.2 mg) in 0.5 mL of dry DMF and 0.2 mL of 2,2-dimethoxypropane (0.2 mL) was stirred with pyridinium *p*-toluenesulfonate (2 mg) under a dry atmosphere at 25 °C for 24 h. The reaction mixture was concentrated to 200 μL and applied to a small ODS column (1 g) and washed quickly with 20 mL of water followed by 25 mL of 1:1 $MeOH/H_2O$. The latter fraction on further purification by reversed-phase HPLC with 3:1 $MeOH/H_2O$ gave 1.1 mg of pure acetonide (**11**).

Method II. A solution of **10** (5 mg) in 2,2-dimethoxypropane (1 mL) was treated with 37% HCl (4 μL) and allowed to stir at 25 °C for 24 h. Finely powdered potassium carbonate (5 mg) was added to the solution, and stirring was continued for 3 h. The resulting solution, at pH 4, was filtered and evaporated by a stream of dry nitrogen gas. The residue was dried in vacuo and purified by reversed-phase HPLC using an eluant of 7:3 $MeOH/H_2O$ to obtain pure **11** (1.8 mg): ^1H NMR (CD_3OD) 5,7-dihydroxy-8-methoxy-6-methyl-8-phenyl-2-octenoic acid 5,7-acetonide (unit *A*) 6.0 (2, dt; 15.5 and 1.4), 6.75 (3, ddd; 15.5, 7.2, and 7.0), 2.36 (4, m), 2.45 (4, m), 3.40 (5, dt; 4.2 and 7.9), 1.97 (6, dqd; 7.9, 7.0, and 4.9), 1.00 (6-Me, d; 7.0), 3.85 (7, dd; 10.0 and 4.9), 4.00 (8, d; 10.0), 3.12 (8-OCH_3, s), 7.25–7.33 (10/11/12/13/14, m), 0.78 (acetonide-CH_3, s), 1.15 (acetonide-CH_3, s); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.58 (2, dd; 8.7 and 6.1), 2.81 (3, dd; 13.8 and 8.7), 3.05 (3, dd; 13.8 and 6.1), 7.24 (5, d; 2.1), 3.82 (7-OCH_3, s), 6.95 (8, d; 8.6), 7.12 (9, dd;

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8.6 and 2.1); 3-amino-2-methylpropionic acid (unit *C*) 2.47 (2, m), 1.02 (2-Me, d; 7.2), 3.20 (3, dd; 13.2 and 7.0), 3.34 (3, dd; 13.2 and 7.0). ^{13}C NMR (CD_3OD) unit *A* 168.1 (1), 126.1 (2), 143.0 (3), 38.0 (4), 75.4 (5), 39.4 (6), 12.0 (6-Me), 73.2 (7), 83.2 (8), 56.4 (8-OCH_3, s), 141.2 (9), 128.98 (10/14), 128.96 (11/13), 128.8 (12), 102.8 (acetonide), 23.8/24.4 (acetonide-CH_3); unit *B* 173.4 (1), 56.2 (2), 38.0 (3), 131.6 (4), 131.9 (5), 123.2 (6), 155.4 (7), 56.6 (7-OCH_3), 113.4 (8), 129.8 (9); unit *C* 173.4 (1), 41.3 (2), 15.5 (2-Me), 43.3 (3).

***R*(+)and *S*(−) Mosher Esters of 9.** Oxalyl chloride (100 μL, 1.14 mmol) was added to a solution of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) (56 mg, 0.24 mmol) and DMF (18 mg, 0.24mmol) in hexane (10 mL) at room temperature. A white precipitate formed immediately. After 1 h, the mixture was filtered and concentrated. About one-tenth of the above residue in 200 mL of CH_2Cl_2 (distilled from P_2O_5) was added to a solution of **9** (3 mg), (dimethylamino)pyridine (2 mg), and triethylamine (5 μL) in 0.5 mL of CH_2Cl_2 and allowed to stand at room temperature for 15 h under argon. 3-(Dimethylamino)-propylamine (5 μL) was added to neutralize excess acid chloride, and the residue obtained after evaporation of the solvent was subjected to reversed-phase HPLC using 4:1 $MeOH/H_2O$ to obtain the (*R*)-(+)-MTPA ester of **9** (2 mg). According to the same experimental procedure, 56 mg of (*S*)-(−)-MTPA and 3.0 mg of 9 were reacted to obtain 2.3 mg of (*S*)-(−)-MTPA ester of **9**. The concentrations of the esters were adjusted to same concentration in CD_3OD, and the ^1H NMR spectra were determined at 500 MHz.

Saponification of 1, 5, and 8. To a solution of 5 mg of **1**, **5**, or **8** in 1 mL of 4:1 acetone/water was added 15 μL of 2 N NaOH. After being stirred at room temperature for 5 h, the reaction mixture was neutralized to pH 7 with 1 N HCl and evaporated. The CH_2Cl_2-soluble material was passed through a small silica cartridge with CH_2Cl_2, 1:1 EtOAc/CH_2Cl_2, and EtOAc. The fraction eluted with EtOAc contained pure cryptophycin F (**12**): ^1H NMR (CD_3OD): 5,7,8-trihydroxy-6-methyl-8-phenyl-2-octenoic acid (unit *A*) 6.07 (2, ddd; 15.5, 1.3, and 1.2), 6.40 (3, dt; 15.5, and 7.3), 2.49 (4, m), 2.60 (4, m), 3.92 (5, ddd; 9.3, 6.7, and 4.5), 1.94 (6, m), 1.07 (6-Me, d; 6.6), 3.61 (7, dd; 8.9, and 7.6), 4.56 (8, d; 7.6), 7.36 (10/14, dd; 7.4, and 1.5), 7.32 (11/13, br t; 7.5), 7.25 (12, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.57 (2, dd; 8.4 and 6.7), 2.83 (3, dd; 13.8 and 8.4), 3.02 (3, dd; 13.8 and 6.7), 7.25 (5, d; 2.1), 3.82 (7-OCH_3, s), 6.95 (8, d; 8.5), 7.12 (9, dd; 8.5 and 2.1); 3-amino-2-methylpropionic acid (unit *C*) 2.54 (2, ddq; 7.0, 6.6, and 7.0), 1.02 (2-Me, d; 7.0), 3.14 (3, dd; 13.5, and 7.0), 3.42 (3, dd; 13.5, and 6.6). Methylation of **12** with diazomethane gave **6**.

The CH_2Cl_2-soluble material was purified by reversed-phase HPLC (Econosil C_{18}, 250 cm × 10 mm, 7:3 $MeOH/H_2O$) to give pure L-leucic acid, R_f = 0.65 for TLC analysis on Chiralplate (Macherey-Nagel) using 1:9 $MeOH/CH_2Cl_2$. The optical rotations for the leucic acid from saponification of **1** and authentic L-leucic acid were identical, $[\alpha]_D$ = −20° (1N NaOH) [reported, $[\alpha]_D$ = −26.3° (1 N NaOH, *c* 1)].

Saponification of Cryptophycin C. To a solution of 3 mg of **3** in 1 mL of 3:1 acetone/H_2O was added 5 μL of 2 N NaOH. After being stirred for 5 h, the reaction mixture was neutralized to pH 7 with 1 N HCl and then evaporated to dryness. The residue was subjected to reversed-phase HPLC on C_{18} with 4:1 $MeOH/H_2O$ to give 2.4 mg of **14**: ^1H NMR (CD_3OD) 5-hydroxy-6-methyl-8-phenyl-2,7-octadienoic acid (unit *A*) 5.98 (2, d; 15.3), 6.78 (3, dt; 15.3 and 7.5), 2.35 (4, m), 3.64 (5, td; 7.2 and 4.8), 2.47 (6, m), 1.14 (6-Me, d; 6.9), 6.22 (7, dd; 15.9 and 8.1), 6.39 (8, d; 15.9), 7.24–7.36 (10/11/12/13/14, m); 3-chloro-4-methoxyphenylalanine (unit *B*) 4.58 (2, dd; 8.8 and 6.3), 2.80 (3, dd; 13.8 and 8.8), 3.05 (3, dd; 13.8 and 6.3), 7.25 (5, d; 2.1), 3.82 (7-OCH_3, s), 6.95 (8, d; 8.4), 7.13 (9, dd; 8.4 and 2.1); 3-amino-2-methylpropionic acid (unit *C*) 2.35 (2, m), 1.02 (2-Me, d; 6.9), 3.18 (3, dd; 13.2, and 6.6), 3.36 (3, dd; 13.2, and 4.5).

Conversion of 3 to 1. Cryptophycin C (**3**, 0.9 mg) was dissolved in 0.5 mL of dry CH_2Cl_2 and treated with 0.7 mg of *m*-chloroperoxybenzoic acid (50–60%) at room temperature for 96 h. The residue obtained after evaporation of the solvent was subjected to reversed-phase HPLC on C_{18} using 1:3 water/MeOH to give 0.1 mg of **1** and 0.3 mg of recovered **3**.

***In Vivo* Antitumor Evaluation.** The methods and endpoints used to evaluate antitumor activity are described elsewhere.[2b] Briefly, tumors were bilaterally implanted as 30–60 mg trocar fragments on day 0. Intravenous treatment was initiated 1–3 days later. MTD total dose values varied with the schedule, lot, and strain of mice.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **1**-**7** in $CDCl_3$ (7 pages). This material is contained in many 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.