Structure Determination, Conformational Analysis, Chemical Stability Studies, and Antitumor Evaluation of the Cryptophycins. Isolation of 18 New Analogs from *Nostoc* sp. Strain GSV 224†

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Abstract: Using a modified isolation procedure devoid of methanol, 18 new cyclic cryptophycins have been isolated from *Nostoc* sp. GSV 224 as minor constituents in addition to cryptophycins-1 (A), -2 (B), -3 (C), and -4 (D). Acyclic cryptophycins are not found, indicating that the previously reported cryptophycins-5 (E methyl ester), -6 (F methyl ester), and -7 (G) are artifacts produced as a consequence of using methanol in the isolation scheme. Seventeen of the new cyclic analogs differ in structure in either one of the two hydroxy acid units, viz. unit A [(5*S*,6*S*,7*R*,8*R*)-7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2(*E*)-octenoic acid for cryptophycin-1 or (5*S*,6*S*)-5-hydroxy-6-methyl-8-phenyl-2(*E*),7(*E*)-octadienoic acid for cryptophycin-3] and unit D [(2*S*)-2-hydroxy-4-methylvaleric acid], or one of the two amino acid units, viz. unit B [(2*R*)-2-amino-3-(3-chloro-4-methoxyphenyl)propionic acid] and unit C [(2*R*)-3-amino-2-methylpropionic acid], found in the cyclic ABCD peptolide. In unit A of cryptophycins-26, -28, -30, and -40, the methyl group on C-6 is missing or the Δ^2-double bond is hydrated. In unit B of cryptophycins-16, -17, -23, -31, -43, and -45, the aromatic ring is phenolic and/or possesses two or zero chlorines. In unit C of cryptophycins-21 and -29, the methyl group on C-2 is missing. In unit D of cryptophycins-18, -19, -49, -50, and -54, a different alkyl group (propyl, isopropyl, or *sec*-butyl) is attached to C-2. Only one of the new analogs, cryptophycin-24, differs in structure for two units by lacking chlorine in unit B and the methyl group in unit C. Revised structures are presented for cryptophycins-5, -6, and -7 and are correlated with cryptophycin-3, the relative stereochemistry of which has been further rigorously established by X-ray crystallography. NOE studies show that the preferred conformations of most cryptophycins in solution differ from the conformation of cryptophycin-3 in the crystal state. Although cryptophycin-1 is relatively stable at pH 7, both in ionic and nonionic media, the ester bond linking units C and D is fairly labile to solvolysis and mild base hydrolysis. Structure−activity relationship studies indicate that the intact macrolide ring, the epoxide group, the chloro and *O*-methyl groups in unit B, and the methyl group in unit C are needed for the *in vivo* activity of cryptophycin-1.

Introduction

Cryptophycins are potent antitumor and antifungal depsipeptides from blue-green algae (cyanobacteria) belonging to the Nostocaceae.[1,2] The first cryptophycin (**1**) was isolated from terrestrial *Nostoc* sp. ATCC 53789 by researchers at Merck and found to be very active against fungi, especially strains of *Cryptococcus*,[1] which frequently infect immunodeficient persons suffering from diseases such as AIDS and cancer. In 1994, we described the isolation of **1**, which we designated cryptophycin A, and six additional cytotoxic analogs from terrestrial *Nostoc* sp. GSV 224. Three of these other cryptophycins were cyclic depsipeptides (**2**−**4**) and three were acyclic depsipeptides (**5**−**7**).[2] We discovered that the peptolides showed significant tumor selective cytotoxicity in the Corbett assay[3] and were equally cytotoxic against drug sensitive and drug resistant tumor cells.[2,4] Preliminary studies have suggested that **1** may operate by irreversibly inhibiting microtubule assembly.[4] Cryptophycin **1** was found to be very effective against subcutaneously transplanted solid tumors in mice when the drug was administered intravenously, displaying excellent activity against five tumors of murine origin, *viz.* colon adenocarcinomas 38 and 51, taxol-

†This paper is dedicated to the memory of Dr. Matthew Suffness.
‡Cancer Research Center of Hawaii.
§ Wayne State University.
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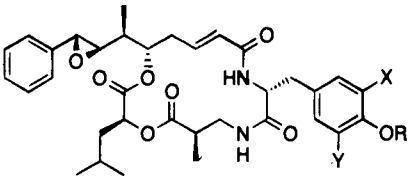
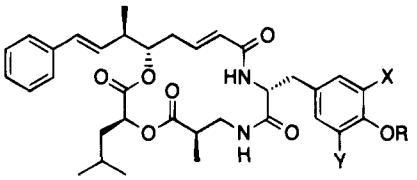
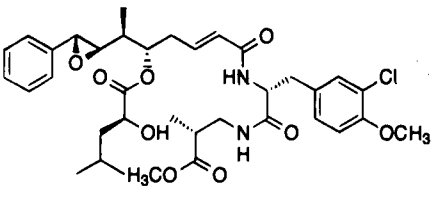


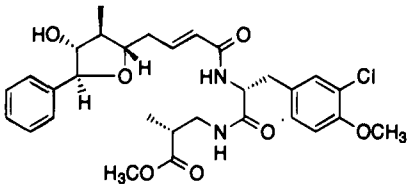
cryptophycin-1 **1** R = CH_3 X = Cl Y = H
cryptophycin-2 **2** R = CH_3 X = H Y = H



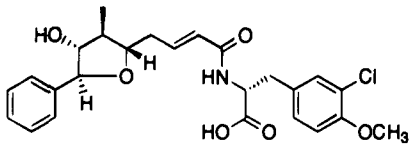
cryptophycin-3 **3** R = CH_3 X = Cl Y = H
cryptophycin-4 **4** R = CH_3 X = H Y = H



cryptophycin-5 **5**



cryptophycin-6 **6**



cryptophycin-7 **7**

sensitive and taxol-resistant mammary adenocarcinoma M16, and pancreatic ductal adenocarcinoma 03, with tumor burden *T/C* values under 10%. It was also active against DMS-273, a highly invasive metastatic human small-cell lung tumor, in the SCID mouse. Prompted by this interesting broad-spectrum antitumor activity, we proceeded to grow *Nostoc* sp. GSV 224 in large scale to procure adequate supplies of **1** and the minor cryptophycins (**2**–**5**) for further *in vivo* evaluation and semisynthesis of analogs for structure–activity relationship (SAR) studies. As a consequence of this expanded investigation, we have now identified 18 new cyclic cryptophycins (**8**–**25**, Chart 1) as minor constituents in *Nostoc* sp. GSV 224. To our chagrin, however, we also found[5] that errors had been made in the stereochemical assignments of **1**, **3**, **5**–**7** and seven semisynthetic analogs derived from **1**, **3**, and **5**.[2] We present here corrected structures for the latter 12 compounds and total structures for **8**-**25**, along with preliminary results on the *in vivo* antitumor activities of five previously described cryptophycins and two of the 18 new cryptophycins.

(5) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479–90.

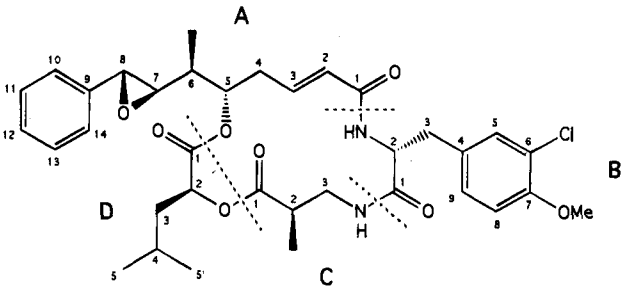


Figure 1. Structure and absolute stereochemistry of cryptophycin-1 and numbering system for the two hydroxy acid units A and D and the two amino acid units B and C. This numbering system is used for the NMR data.

Results and Discussion

Cryptophycin-1 (**1**) is a 3,6,10,16-tetrasubstituted 1,4-dioxa-8,11-diazacyclohexadec-13(*E*)-ene-2,5,9,12-tetrone[6] consisting of units of two hydroxy acids, viz. (5*S*,6*S*,7*R*,8*R*)-7,8-epoxy-5-hydroxy-6-methyl-8-phenyl-2(*E*)-octenoic acid (unit A) and (2*S*)-2-hydroxy-4-methylvaleric acid (unit D; L-leucic acid), and two amino acids, viz. (2*R*)-3-(3-chloro-4-methoxyphenyl)-alanine (unit B) and (2*R*)-3-amino-2-methylpropionic acid (unit C), connected together in a cyclic ABCD sequence as shown in Figure 1.

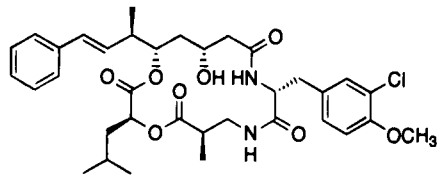
The seven naturally-occurring cryptophycins and artifacts that have already been described in the literature are referred to as cryptophycins A–G in our earlier publications[2,5] and cryptophycins-1, -2, -3, -4, -5, -6, and -7, respectively, in pending patents and in this paper. Seven derivatives (semisynthetic analogs) and degradation products have also been described[2] previously, and these are designated cryptophycins-8 (**26**), -9 (**27**), -10 (**28**), -11, -12 (**29**), -13 (**30**), and -14 (**31**). In addition to the 18 new cryptophycins that have been isolated from *Nostoc* sp. GSV 224 and described in this paper, over 50 more peptolide-type analogs have been prepared in the past year by semisynthesis and total synthesis.[7] Each new cryptophycin that has been isolated from the alga or prepared synthetically has been assigned a number which corresponds roughly to the chronological order of its isolation from the alga or its preparation by synthesis. The 18 new naturally-occurring cryptophycins described in this paper have been designated cryptophycin-16 (**12**), -17 (**15**), -18 (**21**), -19 (**24**), -21 (**18**), -23 (**13**), -24 (**20**), -26 (**9**), -28 (**10**), -29 (**19**), -30 (**8**), -31 (**14**), -40 (**11**), -43 (**16**), -45 (**17**), -49 (**23**), -50 (**22**), and -54 (**25**).

In our 1994 isolation paper[2] we reported that the chlorinated *O*-methyltyrosine unit in cryptophycins-1 (**1**), -3 (**3**), -5 (**5**), -6 (**6**), and -7 (**7**) was L-(*S*), whereas the unsubstituted *O*-methyltyrosine unit in cryptophycins-2 (**2**) and -4 (**4**) was D-(*R*). We should have been more suspicious that an error had been made, as this was an unprecedented finding. The assignments had been made on the basis of Marfey analysis of acid hydrolysates of the cryptophycins. Although total synthesis confirmed the structure of cryptophycin-4, it revealed that cryptophycins-1 and -3 were not the (10*S*)-1,4-dioxa-8,11-diazacyclohexadec-13(*E*)-ene-2,5,9,12-tetrones,[6] but rather the 10*R* stereoisomers depicted in the revised structural drawings

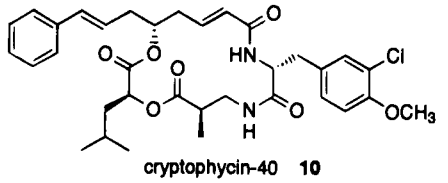
(6) According to *Chemical Abstracts* nomenclature, **1** is [3*S*,6*R*,10*R*,16*S*-(1*S*(2*R*,3*R*))]-10-[(3-chloro-4-methoxyphenyl)methyl]-6-methyl-3-(2-methylpropyl)-16-[1-(3-phenyloxiranyl)ethyl]-1,4-dioxa-8,11-diazacyclohexadec-13(*E*)-ene-2,5,9,12-tetrone and **3** is [3*S*,6*R*,10*R*,16*S*(1*S*)]-10-[(3-chloro-4-methoxyphenyl)methyl]-6-methyl-3-(2-methylpropyl)-16-[1-methyl-3-phenylprop-2(*E*)-enyl]-1,4-dioxa-8,11-diazacyclohexadec-13(*E*)-ene-2,5,9,12-tetrone.

(7) Heltzel, C. E.; Ogino, J.; Golakoti, T.; Patterson, G. M. L.; Moore, R. E.; Larsen, L. K.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. manuscript in preparation.

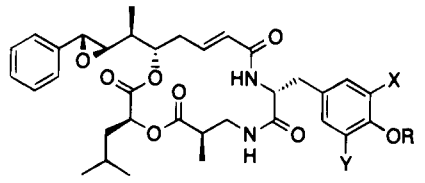
Chart 1



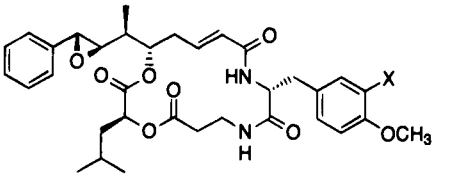
cryptophycin-30 **8**



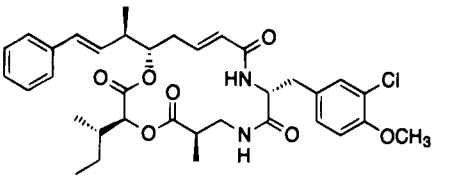
cryptophycin-40 **10**



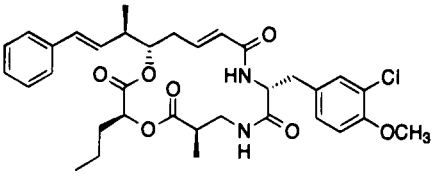
cryptophycin-16 **12** R = H X = Cl Y = H
cryptophycin-23 **13** R = H X = Cl Y = Cl
cryptophycin-31 **14** R = CH_3 X = Cl Y = Cl



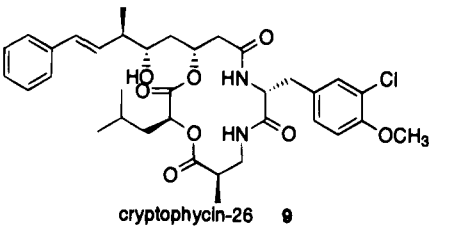
cryptophycin-18 **18** X = Cl
cryptophycin-24 **20** X = H



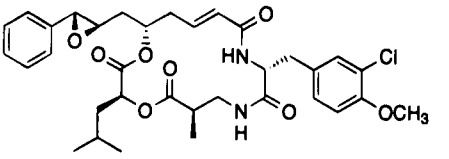
cryptophycin-18 **21**



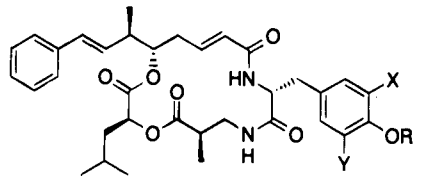
cryptophycin-49 **23**



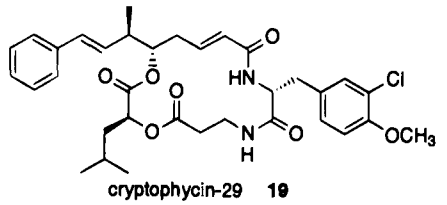
cryptophycin-26 **9**



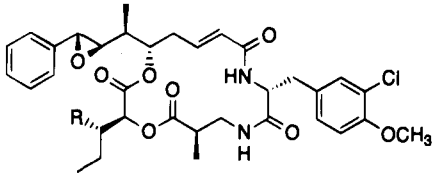
cryptophycin-28 **11**



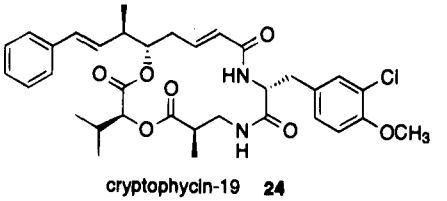
cryptophycin-17 **15** R = H X = Cl Y = H
cryptophycin-43 **16** R = H X = H Y = H
cryptophycin-45 **17** R = H X = Cl Y = Cl



cryptophycin-29 **19**



cryptophycin-50 **22** R = H
cryptophycin-54 **25** R = CH_3



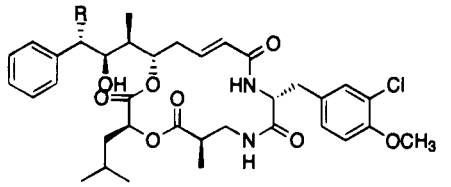
cryptophycin-19 **24**

1 and **3**, respectively.[5] When we repeated the acid hydrolysis of **1** on a larger scale under milder conditions (constant boiling HCl at 90 °C for 12 h), D-3-(3-chloro-4-methoxyphenyl)alanine (CMPHE), D-3-amino-2-methylpropionic acid (AMPA), and L-leucic acid (LA) were obtained in sufficient amounts to determine their optical properties. The D-CMPHE, D-AMPA, and L-LA exhibited specific rotations that were comparable with those of authentic samples.[8-10] The results confirmed our original assignments of absolute configuration for units C and D, but showed that we had erred in the unit B assignment. Optically active 3-benzyl-2,4-dimethyl-2-cyclopentenone (**32**) was also obtained in good yield in the large scale acid hydrolysis, obviously from degradation of unit A in **1**.

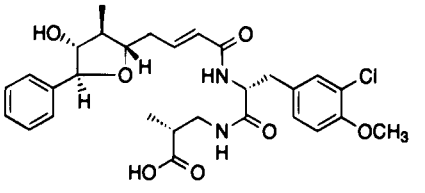
In our earlier study,[2] the D-CMPHE had undergone appreciable racemization under the more vigorous acid hydrolysis conditions (HCl at 110 °C for 12 h). As a consequence, a significant peak had been observed in the Marfey HPLC chromatogram of the acid hydrolysate which matched the retention time of a commercial L-CMPHE standard. A larger peak with a longer (4 min longer) retention time was also present; however, it did not coincide with the retention time that had been found for a standard believed to be the D isomer. In an attempt to racemize the L-CMPHE standard, we had treated the amino acid with a mixture of acetic anhydride and acetic acid heated to reflux for 30 min.[11] Indeed, a 3:2 mixture of L-CMPHE and another compound (t_R 49.2 min), which we presumed was D-CMPHE, had been obtained since this proce-

(8) Guroff, G. *Biochem. Biophys. Res. Commun.* **1966**, *25*, 622–8.
(9) (a) Kakimoto, Y.; Armsrong, M. D. *J. Biol. Chem.* **1961**, *236*, 3283–6. (b) Pollock, G. *Anal. Biochem.* **1974**, *57*, 82–8.
(10) (a) Fuganti, C.; Ghiringhelli, D. *Gazz. Chim. Ital.* **1969**, *99*, 316–22. (b) Mori, K. *Tetrahedron* **1976**, *32*, 1101–6.

Chart 2



cryptophycin-8 **26** R = Cl
cryptophycin-9 **27** R = OCH_3



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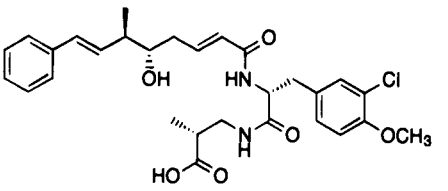

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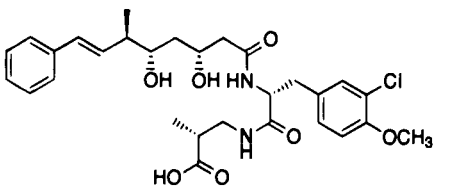

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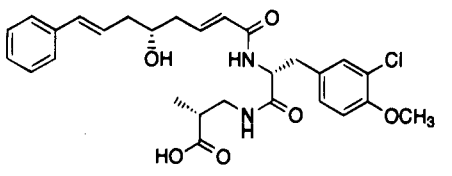

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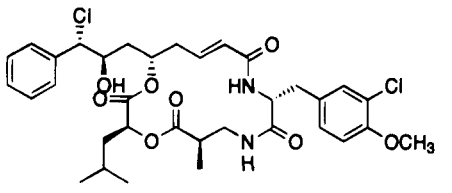

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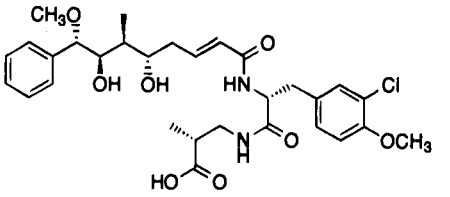
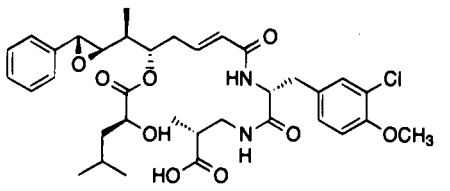


cryptophycin-10 **28**



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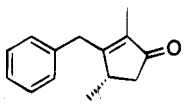

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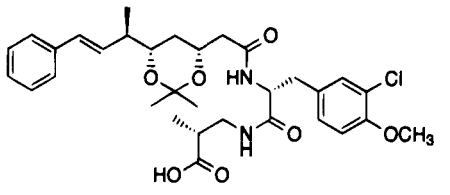

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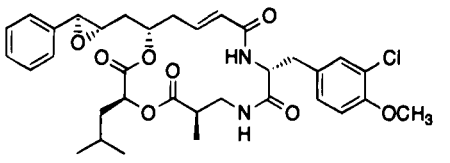
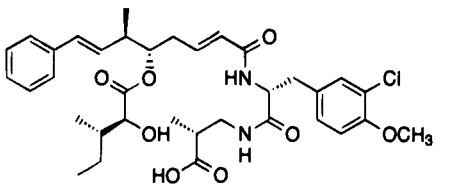


cryptophycin-107 **36**



38

dure had been used before to racemize α-amino acids. After discovering through synthesis that we had made an error in the absolute stereochemistry of the CMPHE unit, we isolated the compound eluting at 49.2 min and showed that it was the di-FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) derivative of L-3-(3-chloro-4-hydroxyphenyl)alanine. Demethylation of L-CMPHE to the phenol had occurred instead of racemization. Marfey analysis using an authentic D-CMPHE standard (t_R 48.4 min) synthesized from D-tyrosine provided the final proof that we had erred in the assignment of configuration for unit B in cryptophycins-1 (**1**), -3 (**3**), -5 (**5**), -6 (**6**), -7 (**7**), -8 (**26**), -9 (**27**), -10 (**28**), -11, -12 (**29**), -13 (**30**), and -14 (**31**) (Chart 2). The CMPHE unit in these 12 compounds was D-(*R*), the same absolute stereochemistry found for the *O*-methyltyrosine unit in cryptophycins-2 (**2**) and -4 (**4**).

Further rigorous proof for the revised stereochemistry of the monochlorocryptophycins was obtained from the X-ray crystal structure of **3** shown in Figure 2 and discussed below in the Conformational Analysis section.

In reexamining the spectral data for **6**, **7**, and **29**, we also found that their gross structures had been depicted incorrectly as triols in our earlier paper.[2] Firstly, the FAB mass spectra of these three acyclic cryptophycins suggested that their molecular weights were 18 mass units less than the ones required for triol structures. Secondly, the ^{13}C NMR spectra provided no evidence for the presence of three hydroxyl groups on an acyclic carbon chain. The chemical shifts for the three oxygen-bearing carbons in unit A of **6** and **7** were all 82−86 ppm. By

(11) (a) Bergmann, M.; Zervas, L. *Biochem. Z.* **1928**, *203*, 280. (b) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 3, p 2364.

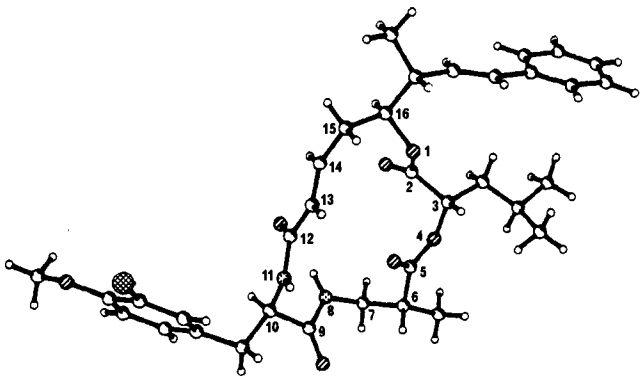


Figure 2.

comparison, the hydroxyl-bearing methine carbons in **28** resonated at 74.1 and 74.5 ppm, whereas the methoxyl-bearing methine carbon resonated at 85.9 ppm.[2] Thirdly, the ^{1}H NMR spectra exhibited quite different chemical shifts and coupling patterns when compared with the ones for **28**.[2] The data fit much better for hydroxytetrahydrofuran structures as shown in **6**, **7**, and **29**. Additional chemical and spectral studies proved that they were hydroxytetrahydrofurans. For example, when the ^{13}C NMR spectra of **29** in CD_3OH and CD_3OD were compared, the methine signal assigned to C-7 in unit A (85.9 ppm in CD_3OD; ^1J correlated with a ^{1}H signal at 3.61 ppm) revealed a $\Delta\delta_C$ of 0.1 ppm in the two solvents (see the Experimental Section), strongly suggesting that a hydroxyl group was attached to the methine. Furthermore, **29** formed a mono-*O*-acetate derivative and ^{1}H NMR analysis clearly showed that the acetylated oxygen was attached to C-7 of unit A ($\Delta\delta_H$ 1.39 ppm). Finally, the HMBC spectrum of **29** showed a cross peak for $^3J_{H,C}$ coupling between 8-H and C-5, but not 5-H and C-8, of unit A, entirely consistent with the trisubstituted tetrahydrofuran structure and dihedral angles of 141° and 87° calculated for H−C8−O−C5 and C8−O−C5−H, respectively, in the energy-minimized conformation.

Isolation and Structure Determination. Concerning the cultures of *Nostoc* sp. GSV 224 that have been produced in our laboratory over the past 4 years, cryptophycins **1**−**4** have always been found in the algal extracts. Acyclic cryptophycins **5**−**7**, however, have proven to be artifacts formed as a consequence of using methanol in the isolation scheme. When methanol was eliminated from the extraction and chromatography steps, **5**−**7** and other acyclic cryptophycins[12] could not be found. Only peptolides were isolated, most of which were new, albeit minor, components. The acyclic artifacts **5** and **6** appeared to involve the methanolysis of **1**; however, the origin of **7** was unclear.

In a typical revised isolation procedure, the dried alga was extracted with 5:1 acetonitrile/dichloromethane and the concentrated extract was fractionated by reversed-phase flash chromatography with water/acetonitrile mixtures. All of the cryptophycins were eluted with 35% H_2O/CH_3CN, and this fraction was further subjected to reversed phase HPLC and separated into a number of subfractions. Repeated chromatography of each subfraction by normal phase and/or reversed phase HPLC led to the four known macrolides (**1**−**4**) and the 18 new analogs (**8**−25). The gross structures and stereochemistries of **8**−**25** were established in a straightforward manner using a combination of chemical and spectral techniques.

Two types of cryptophycins predominated, viz. styrene epoxides and the corresponding styrenes. When the structures

(12) For example, acyclic artifacts of cryptophycin-2 and cryptophycin-21 that are related to **5** are produced when MeOH is used in the isolation procedure (Lee, D. Y. G. Unpublished results).

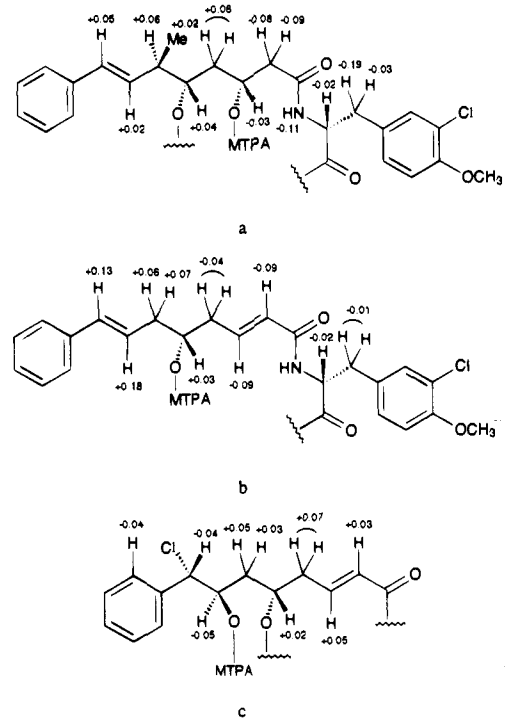


Figure 3. ΔS ($\delta_S - \delta_R$) values in ppm obtained at 500 MHz for the (*R*)- and (*S*)-MTPA esters of **8** (a), **35** (b), and **37** (c).

of the minor epoxides and styrenes were compared with **1** and **3**, respectively, differences were found in only *one unit* for 17 of the new analogs. Only one analog, cryptophycin-24 (**20**), differed in structure for two units.

Analogs Differing in Unit A. Four of the 18 new cryptophycins differed in unit A. Two compounds, cryptophycin-30 (**8**) and -26 (**9**), were styrenes lacking the Δ^2-double bond, whereas the other two compounds, cryptophycin-28 (**10**) and -40 (**11**), were the styrene and corresponding styrene epoxide that lacked the methyl group on C-6.

Cryptophycin-30 (**8**) had the formula $C_{35}H_{45}ClN_2O_8$ based on high-resolution mass spectrometry. Spectrally, it was clearly different from the other cryptophycins. The NMR signals associated with the α,β-unsaturated amide moiety in unit A were missing, and instead signals were present for a secondary alcohol functionality at C3 and two protons on C2. The C3 methine and hydroxyl protons resonated at 3.89 and 2.51 ppm, respectively, and the C2 methylene protons absorbed at 2.25 and 2.64 ppm and showed a characteristic geminal coupling of −16 Hz, indicative of a methylene adjacent to a carbonyl group. The rest of the gross structure was concluded to be the same as that in **3** from two-dimensional NMR spectral analysis. A two-step conversion of **8** to **3**, via tosylation and subsequent E2 elimination of *p*-toluenesulfonic acid by treatment with DBU, confirmed the structure and provided rigorous proof for the absolute stereochemistry of all the chiral centers in the molecule except for C-3 in unit A. The absolute stereochemistry at this carbon was assigned by Mosher analysis.[13] The *O*-(*R*)- and -(*S*)-MTPA derivatives of **8** were prepared and $\Delta\delta$ values ($\delta_S - \delta_R$) were determined at 500 MHz for all of the assignable protons. Positive $\Delta\delta$ values were found for all of the protons on the C4−C14 side of the MTPA plane, whereas negative values were found for protons on the C2 side (Figure 3a). This meant that C3 in unit A was *R*. To further confirm the absolute stereo-

(13) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−9. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−6. (c) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939−42.

chemistry at C3, **8** was saponified to a diol **33** and converted into an acetonide **34**. The chemical shifts of the acetonide ketal (100.2 ppm) and methyl carbons (20.0 and 30.4 ppm for axial and equatorial methyls, respectively)[14] and the diaxial-type coupling constants (11.4 Hz) for the two methine protons in the acetonide ring[15] indicated that **34** was an acetonide of a *cis*-1,3 diol. As expected for a chair conformation for this *syn*-acetonide, the H-3 signal at 4.21 ppm and the H-5 signal at 3.83 ppm exhibited strong NOEs to the axial methyl signal at 1.41 ppm. These data further supported the absolute stereochemistry of unit A in **8** as being 3*R*,5*S*,6*R*.

Cryptophycin-26 (**9**) was found to have the same molecular formula as **8**, $C_{35}H_{45}ClN_2O_8$, by high-resolution EI mass spectrometry. Inspection of the NMR data revealed that **9** consisted of the same amino and hydroxy acid units as **8**, including a 3,5-dihydroxy-6-methyl-8-phenyl-7-octenoic acid derived unit A. The methine protons on the carbons bearing the ester and hydroxyl functionalities in unit A resonated at 5.46 and 3.61 ppm, respectively, and analysis of the COSY, HMQC, and HMBC data clearly showed that the ester function was attached to C3 and the hydroxyl group resided on C5. This meant that **9** was a 14-membered peptolide, the first representative of this type among the cryptophycin congeners. The relative and absolute stereochemistries of **9** and **8** were shown to be identical by the following interconversions, *viz.* ring contraction[16] of **8** to **9** in the presence of thionyl chloride and pyridine and ring expansion[17] of **9** to **8** with a catalytic amount of a strong acid (HBr) in 1,2-dimethoxyethane. The stereochemical assignments were further confirmed by saponification of **9** and **8**, which furnished the same reaction products, **33** and L-leucic acid.

Cryptophycin-28 (**10**) had the molecular composition $C_{34}H_{41}ClN_2O_7$. Its ^{1}H NMR spectrum exhibited some similarities with those of **3**, showing signals for the same B, C, and D units, but an A unit which lacked the methyl group on C-6. The characteristic doublet signal for this methyl group was missing and replaced by a signal for a second proton on C-6. The EI mass spectrum supported this difference in structure with a strong fragment ion at *m/z* 213, assignable to a C_6H_5-CH=CHCH=CHCH$_2$CH=CHC(OH)=NH ion generated from unit A of **10** in the same manner that the *m/z* 227 ion is formed in the EI mass spectra of cryptophycins **3** and **4**.[2] To determine the absolute stereochemistry of C-5 in unit A, **10** was saponified to hydroxy acid **35**, which was then converted into (*R*)- and (*S*)-MTPA esters. Mosher analysis clearly showed that C-5 was *R*. As depicted in Figure 3b, protons on the C4−C1 side of the CHOMTPA plane showed negative $\Delta\delta$ ($\delta_S-\delta_R$) values, whereas positive $\Delta\delta$ values were found for protons on the C6−C8 side. Marfey[18] analysis of the CMPHE in the acid hydrolysate indicated that the absolute configuration of unit B was D-(*R*). Chiral GC analysis,[19] coupled with mass spectrometry, of the AMPA in the acid hydrolysate as previously described[2] indicated that unit C was D-(*R*). Chiral TLC analysis[20] of the leucic acid in the acid hydrolysate indicated that unit D was L-(*S*).

Cryptophycin-40 (**11**), $C_{34}H_{41}ClN_2O_8$, was the second least abundant peptolide in the alga. The ^{1}H NMR spectrum of **11** was very similar to that of **10**, the major difference being the replacement of olefinic-type signals for 7-H and 8-H in unit A of **10** by epoxide-type resonances for **11**. The C7 epoxy methine proton showed vicinal coupling to three protons, viz. 8-H and two protons on C-6, in agreement with the absence of a methyl group on C-6. The structure was confirmed and the absolute stereochemistry established for all the chiral carbons except C-7 and C-8 in unit A by epoxidation of **10** to 2:1 mixture of **11** and the *S,S*-epoxide **36** with *m*-chloroperoxybenzoic acid. The absolute stereochemistry of the epoxide ring in **11** was shown to be *R,R* by Mosher analysis of chlorohydrin **37** (Figure 3c).

Analogs Differing in Unit B. Six of the 18 new cryptophycins differed in unit B. Five compounds, cryptophycin-16 (**12**) -23 (**13**), -17 (**15**), -43 (**16**), and -45 (**17**), were phenols possessing two, one, or zero chlorine atoms on the tyrosine ring whereas cryptophycin-31 (**14**) was a styrene epoxide possessing an additional chlorine atom *ortho* to the *O*-methyl substituent.

Cryptophycin-16 (**12**), the most polar of the new compounds, had the molecular formula $C_{34}H_{41}ClN_2O_8$ based on its high resolution EI mass spectrum. Its ^{1}H NMR spectrum bore a close resemblance to that of **1**, but the *O*-methyl signal was missing and an exchangeable proton signal was present at 5.61 ppm. The remaining proton signals had chemical shifts and coupling patterns that were virtually identical with those found for **1**, indicating that **12** differed from **1** by only the absence of the *O*-methyl group in unit B. This difference in structure was corroborated by the ^{13}C NMR spectrum which also lacked an *O*-methyl signal and displayed an expected upfield shift of the signal for C7 in unit B from 154.0 ppm for **1** to 150.4 ppm for **12**. Conversion of **12** to **1** by treatment with methyl iodide/potassium carbonate in acetone confirmed the structure and furthermore established that **12** had the same absolute stereochemistry as **1**.

Cryptophycin-23 (**13**) exhibited a molecular ion cluster in its EI mass spectrum that was consistent with the formula $C_{34}H_{40}Cl_2N_2O_8$. Comparison of spectral data suggested that **13** was closely related to **12**. The resonances for the chlorotyrosine unit were missing in the ^{1}H NMR spectrum, and instead a 2H singlet was present at 7.13 ppm, suggesting that unit B in **13** was a 3-(3,5-dichloro-4-hydroxyphenyl)alanine residue. The presence of the dichlorotyrosine unit was confirmed by acid hydrolysis. The remainder of the structure was concluded to be the same as that in **12** from analysis of the COSY, HMQC, and HMBC spectral data. The chemical shifts and coupling constants for the protons on the C1−C8 segment of the unit A of **12** and **13** were virtually identical, strongly suggesting that unit A in the two compounds had the same relative and absolute stereochemistry. Again, Marfey analysis[18] of the CMPHE, chiral GC−MS analysis[19] of the AMPA, and chiral TLC analysis[20] of the LA in the acid hydrolysate rigorously established the absolute configurations of unit B as D-(*R*), unit C as D-(*R*), and unit D as L-(*S*).

Cryptophycin-31 (**14**), $C_{35}H_{42}Cl_2N_2O_8$, was identified as the *O*-methyl analog of **13**. Appropriate ^{1}H and ^{13}C NMR signals were observed at 3.87 and 60.6 ppm, respectively, for the methoxy group. Conversion of **13** into **14** with methyl iodide in the presence of potassium carbonate in acetone confirmed the proposed structure and established that the two compounds had the same absolute configuration.

Cryptophycin-17 (**15**), $C_{34}H_{41}ClN_2O_7$, exhibited significant structural similarities with those of the known compound **3**. Apart from the absence of an *O*-methyl signal and the presence of a broad signal at 5.87 ppm for an exchangeable proton, the

(14) (a) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945−8. (b) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511−5.

(15) Marshall, J. A.; Crute, T. D., III; Hsi, J. D. *J. Org. Chem.* **1992**, *57*, 115−23.

(16) For an example of ring contraction see: Nelson, F.; C.; Stachel, S. J.; Mattes, J. F. *Tetrahedron Lett.* **1994**, *41*, 7557−60.

(17) For an example of ring expansion see: Hanessian, S.; Meng, Q.; Oliver, E. *Tetrahedron Lett.* **1994**, *35*, 5393−96.

(18) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−6.

(19) Frank, H.; Nicholson, G. J.; Bayer, E. *J. Chromatogr. Sci.* **1977**, *15*, 174−6.

(20) Gunther, K. *J. Chromatogr.* **1988**, *448*, 11−30.

^{1}H NMR spectra of **15** and **3** were virtually identical, indicating that **15** possessed a phenolic OH in lieu of the *O*-methyl group in **3**. Conversion of **15** to **3** with methyl iodide/potassium carbonate confirmed the structure and provided proof for the absolute stereochemistry.

Cryptophycin-43 (**16**) was found to have the molecular formula $C_{34}H_{42}N_2O_7$. Comparison of spectral data for **16** and **4** indicated that unit B in **16** was a tyrosine residue. The chemical shifts and coupling patterns for units A–C were essentially identical, which strongly suggested that the two compounds had the same stereochemistry.

Cryptophycin-45 (**17**) was analyzed for $C_{34}H_{40}Cl_2N_2O_7$ by HREIMS and exhibited a ^{1}H NMR spectrum that was similar to that of cryptophycin-23 (**13**). The epoxy methine signals were absent, however, and signals were present at 6.01 and 6.42 ppm for a *trans* double bond between C7 and C8 in unit A. The absolute stereochemistries of **17** and **13** were identical, since **13** could be converted into **17** by deepoxidation with titanocene dichloride.[21]

Analogs Differing in Unit C. Two of the 18 new cryptophycins differed in unit C. Cryptophycin-21 (**18**) and -29 (**19**) were found to be the styrene epoxide and corresponding styrene which lacked the methyl group on C-2.

Cryptophycin-21 (**18**) had the molecular composition $C_{34}H_{41}ClN_2O_8$ by high-resolution EIMS. Its proton NMR spectrum was similar to the one for **1**, the only difference being that the signal for the methyl group on C2 in unit C was missing and a signal for two protons on C2 was present. This meant that unit C had to be a N-substituted 3-aminopropanoyl unit. GC–MS analysis confirmed this sole difference in structure by revealing the presence of β-alanine rather than D-AMPA in the acid hydrolysate. Marfey and chiral TLC analyses of the acid hydrolysate further confirmed that unit B was D-(*R*) and unit D was L-(*S*).

Cryptophycin-29 (**19**), $C_{34}H_{41}ClN_2O_7$, is related to **3** in the same way that **18** is related to **1**. The ^{1}H NMR spectrum for **19** was the same as the one for **18**, except that the epoxy methine signals for **18** were replaced by olefinic signals characteristic of a trans double bond between C-7 and C-8 in unit A for **19**. Conversion of **18** to **19** by deepoxidation with titanocene dichloride provided rigorous proof that these two compounds had this close relationship and the same absolute stereochemistries.

Analogs Differing in Unit B and Unit C. Cryptophycin-24 (**20**) was found to be a styrene epoxide lacking the chlorine group in unit B and the methyl group on C-2 of unit C. The compound was analyzed for $C_{34}H_{42}N_2O_8$ by high-resolution mass spectrometry. Detailed NMR analysis characterized it as a desmethyl analog of cryptophycin-2 (**2**), whereby the methyl group on C-2 of unit C was absent. Analysis of the acid hydrolysate confirmed that unit C was a N-substituted 3-aminopropanoyl group and that units B and D possessed D-(*R*) and L-(*S*) configurations, respectively. Since the spectral patterns for the unit A protons of **20** and **2** were comparable, unit A of **20** and **2** had to have the same stereochemistry. Interestingly, **20** is identical with arenastatin A, a minor constituent of the Japanese sponge *Dysidea arenaria*.[22] The possibility that arenastatin A might originate from a cyanobacterial symbiont[23] is being investigated.

Analogs Differing in Unit D. Five of the 18 new analogs, cryptophycin-18 (**21**), -50 (**22**), -49 (**23**), -19 (**24**), and -54 (**25**), differed in unit D. In lieu of an isobutyl group, **22** and **23** have a *n*-propyl group, **24** has an isopropyl group, and **21** and **25** have a *sec*-butyl group attached to C-2.

Cryptophycin-18 (**21**) was found to have the same molecular formula, $C_{35}H_{43}ClN_2O_7$, as **3** by mass spectrometry. Its ^{1}H NMR spectrum exhibited the same signals for units A, B, and C, but clearly different ones for unit D which was shown to be a 2-(acyloxy)-3-methylpentanoyl unit. Mild base hydrolysis of **21** resulted in selective cleavage of the C–D ester bond and the formation of hydroxy acid **38**. Treatment of **21** with dilute aqueous sodium hydroxide, however, yielded **31**, the same degradation product that had been obtained from saponification of **3**,[2] confirming that **21** had the same A, B, and C units as **3**. Isoleucic acid was also formed from saponification of **21**, whereas leucic acid had been produced from **3**. The isoleucic acid showed ^{1}H and ^{13}C spectra that were essentially identical with those of D-(*R,R*)- and L-(*S,S*)-2-hydroxy-3-methylvaleric acid, but significantly different from those of the *allo* (*RS* or *SR*) diastereomers. Unit D in **21** had to have the *S,S* configuration, since the isoleucic acid (Na salt) from **21** was levorotatory[24] and identical with authentic L-(*S,S*)-2-hydroxy-3-methylvaleric acid by chiral TLC analysis.

Cryptophycin-50 (**22**) and cryptophycin-49 (**23**) had the molecular formulas $C_{34}H_{41}ClN_2O_8$ and $C_{34}H_{41}ClN_2O_7$, respectively, by mass spectrometry The ^{1}H NMR spectrum of **22** was very similar to that of **1**, the only difference being in the signals for unit D. NMR analysis indicated that unit D was a 2-(acyloxy)pentanoyl unit. The relative stereochemistry in **22** was concluded to be same as that in **1** based on the strikingly close resemblance of the NMR data. Moreover, the two compounds exhibited essentially identical $[\alpha]_D$'s, +32.0° in $CHCl_3$ for **22** compared with +36.4° in $CHCl_3$ for **1**, strongly suggesting identical absolute stereochemistries. Marfey and chiral GC–MS analyses of the acid hydrolysate of **22** confirmed that unit B was D-(*R*) and unit C was D-(*R*). Unit D in **22** has the L-(*S*) configuration since the 2-hydroxyvaleric acid from **22** was identical with an authentic standard by chiral TLC analysis. Comparison of the spectral data for **22** and **23** indicated that these two compounds shared the same structural relationship that compound **1** had with **3**.

Cryptophycin-19 (**24**) showed the molecular composition $C_{34}H_{41}ClN_2O_7$, CH_2 less than the formulas for **21** and **3**. NMR analysis indicated that units A, B, and C in **24** were the same as those present in **21** and **3**, but unit D was different. The COSY spectrum, for example, shows that the signal for the C2 proton of unit D is coupled to a methine proton signal at 2.09 ppm, which in turn is coupled to two methyl proton signals at 0.84 and 0.95 ppm. From these data unit D in **24** is a 2-acyloxy-3-methylbutanoyl unit. Furthermore, unit D is L-(*S*) since chiral TLC analysis shows that the 2-hydroxy-3-methylbutyric acid in the acid hydrolysate is L-(*S*).[25] The stereochemistry of unit A in **24** should be the same as that in **21** and **3**, as these compounds showed remarkably similar chemical shifts and coupling constants for all the protons of unit A. Unit B was

(21) RajanBabu, T. V.; Nugent, W. A. *J. Am. Chem. Soc.* **1994**, *116*, 986–97.

(22) (a) Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.*, **1994**, *35*, 7969–72. (b) Kobayashi, M.; Aoki, S.; Ohyabu,, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* (Abstracts of Symposium on the Chemistry of Natural Products, Hiroshima) **1994**, *36*, 744–751. (c) Kobayashi, M.; Kurosu, M.; Ohyabu, N.; Wang, W.; Fujii, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2196–8. (d) Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2394–6.

(23) For examples of secondary metabolites from cyanobacterial symbionts of sponges belonging to the genus *Dysidea*, see: (a) Faulkner, D. J.; He, H-Y.; Unson, M. D.; Bewley, C. A. *Gazz. Chim. Ital.* **1993**, *123*, 301–308. (b) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349–353. (c) Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336–6343.

(24) Winitz, M.; Bloch-Frankenthal, L.; Izumiya, N.; Birnbaum, S. M.; Baker, C. G.; Greenstein, J. P. *J. Am. Chem. Soc.* **1956**, *78*, 2423–30.

(25) Baker, C. G.; Meister, A. *J. Am. Chem. Soc.* **1951**, *73*, 1336.

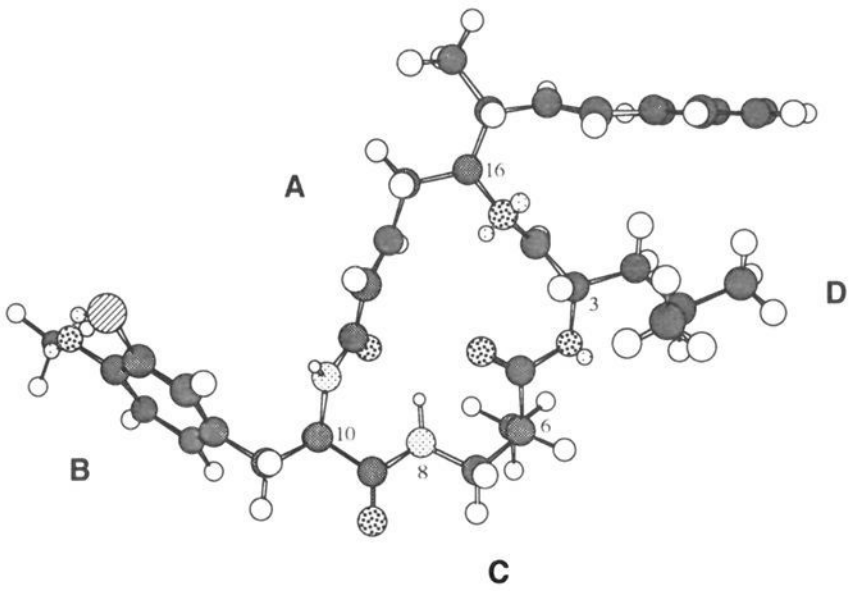


Figure 4. Energy-minimized structure of cryptophycin-3 (**3**) representing the predominant conformer present in dimethyl sulfoxide or chloroform solution. The conformations shown for the peptolide ring and each of the side chains attached to C-3, C-10, and C-16 are supported by NOE and coupling constant data. The peptolide ring conformation differs from the crystal structure in unit C. The methyl carbon attached to C-6 is *syn* to N-8 in solution and *anti* to N-8 in the crystal structure. The side chain conformations depicted are the same as the ones observed in the crystal structure. Nonbonding pairs of electrons are shown on O-1, O-4, and the methoxyl oxygens.

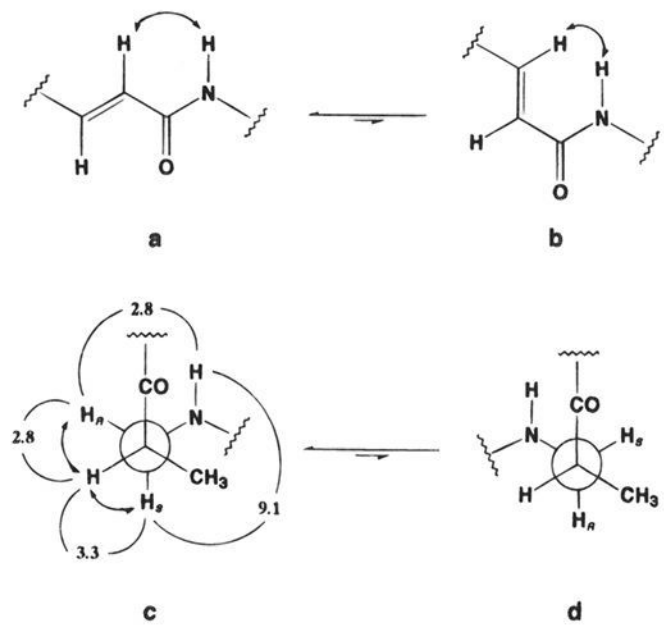


Figure 5. Conformations of the enecarbonyl system in unit A of the cryptophycins in dimethyl sulfoxide (a), chloroform at −50 °C (a) and chloroform at 25 °C (a and b) as shown by NOE data. Conformations of the C1′-C2-C3-N chain of unit C of the cryptophycins in dimethyl sulfoxide (c), chloroform at −50 °C (c) and chloroform at 25 °C (c and d) as shown by NOE and coupling data (*J*-values in DMSO shown).

assigned the D absolute stereochemistry by Marfey analysis of the acid hydrolysate. The D-configuration had also been assigned to the β-amino acid as the derivatized 3-amino-2-methylpropionic acid (AMPA) from **24** had a retention time (t_R) on Chirasil-Val GC column that was identical with the one for the derivatized D-(*R*)-AMPA.

Cryptophycin-54 (**25**), a trace constituent in the alga, was not isolated in sufficient quantity for independent structure studies. Its presence in the alga was established by comparing the HPLC chromatograms of semisynthetic **25** and the crude mixture of cryptophycins. Cryptophycin-54 had been prepared, along with the *S,S*-epoxide **39**, by epoxidation of **21** with *m*-chloroperoxybenzoic acid. Once the HPLC fraction that contained **25** had been identified, an adequate amount of natural **25** (100 μg) was then isolated for direct ^{1}H NMR spectral comparison with the semisynthetic material.

Conformational Analysis. The conformation of cryptophycin-3 (**3**) in the X-ray crystal structure (Figure 2) appears normal, except that the α,β-unsaturated carbonyl system (O=C1C2=C3) in unit A is cis planar rather than trans planar. In simple models such as acrolein, the conformation of lowest energy is trans planar.[26] The two carbons attached to each ester and amide group are *anti* to each other and the β-carbon in each of the side chains in units A, B, and D is *anti* to a carbon atom rather than a heteroatom in the peptolide ring. The carbon chains for C3−C4−C5−C6−C7 in unit A, C1−C2−C3−C4 in unit B, and C1−C2−C3−C4−C5 in unit D are fully extended and essentially planar. The C1′−C2−C3−N segment of unit C is also planar where C1′ is *anti* to N.

In dimethyl sulfoxide-d_6 (DMSO) at 25 °C, the peptolide ring of **3** exists predominantly in a conformation (Figure 4) having only one major difference compared with the crystal structure (see next paragraph). The enecarbonyl system in unit A is the same, i.e., cis planar, based upon the observance of a strong NOE signal between the amide proton in unit B and 2-H in unit A (Figure 5a). Significant NOE signals between 2-H and 4-$H_{pro\text{-}R}$, 3-H and 4-$H_{pro\text{-}S}$ and 3-H and 5-H further support the cis planar conformation. In chloroform-*d* at 25 °C, however, NOE signals of comparable magnitudes are seen between N-H in unit B and each of the protons on C-2 and C-3 in unit A, suggesting that the enecarbonyl system exists in two conformations, one of which is cis planar and other trans planar (Figure 5b). If true, then these two conformations have to be rapidly interconverting as doubled signals are not observed. As the temperature is cooled to −50 °C, the NOE favoring the cis planar conformation grows stronger and the NOE for the trans planar conformation becomes weaker. The signals (i.e., δ and *J* values) for the nonexchangeable protons in the vicinity of the enecarbonyl system, however, do not change significantly between 25 and −50 °C. The data clearly favor the cis planar conformation as the predominant one in $CDCl_3$. The NOE detected between NH in unit B and 3-H in unit A is the only one that supports the presence of a minor trans planar conformation in $CDCl_3$.

In DMSO, the C1′−C2−C3−N segment of unit C appears to be predominantly nonplanar (gauche) where C1′ is *syn* to N, a conformation that is generated from the one in the crystal structure by a 60° counterclockwise rotation of C-2 (C-6 in Figure 2) around the C1−C2 bond (C5−C6 in Figure 2). In this conformation the dihedral angles between 2-H and the two protons on C-3 are ~60° (Figure 5c) as reflected by couplings of 3.3 and 2.5 Hz for $J_{2,3pro\text{-}S}$ and $J_{2,3pro\text{-}R}$, respectively, and strong NOE signals between 2-H and the two protons on C-3. Moreover, couplings of 9.1 and 2.8 Hz for $J_{NH,3pro\text{-}S}$ and $J_{NH,3pro\text{-}R}$, respectively, are consistent with the dihedral angles of 180° and 60° between the amide proton and the two protons on C-3. If unit C had the crystal-state conformation in DMSO, where the dihedral angles were 180° between 2-H and 3-$H_{pro\text{-}S}$ and 60° between 2-H and 3-$H_{pro\text{-}R}$ (Figure 5d), one would have seen a relatively large coupling (~10 Hz) between 2-H and 3-$H_{pro\text{-}S}$, a small coupling (~3 Hz) between 2-H and 3-$H_{pro\text{-}R}$, and only one NOE signal, namely the one between 2-H and 3-$H_{pro\text{-}R}$. Large and small couplings would still have been observed between NH and the protons on C-3; however, the *J* values would be reversed, i.e., 2.8 Hz for $J_{NH,3pro\text{-}S}$ and 9.1 Hz for $J_{NH,3pro\text{-}R}$.

In chloroform at 25 °C, however, the C1′−C2−C3−N segment of unit C must exist in the nonplanar (*syn*) and planar

(26) MO calculations of acrolein, for example, show that the trans planar conformation has the lowest energy and suggest that the cis planar rather than the gauche conformation has the second minimum. Experimental evidence indicates that the energy difference is 2.0−2.06 kcal/mol. Hebre, V. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley-Interscience: New York, 1986; pp 268−9.

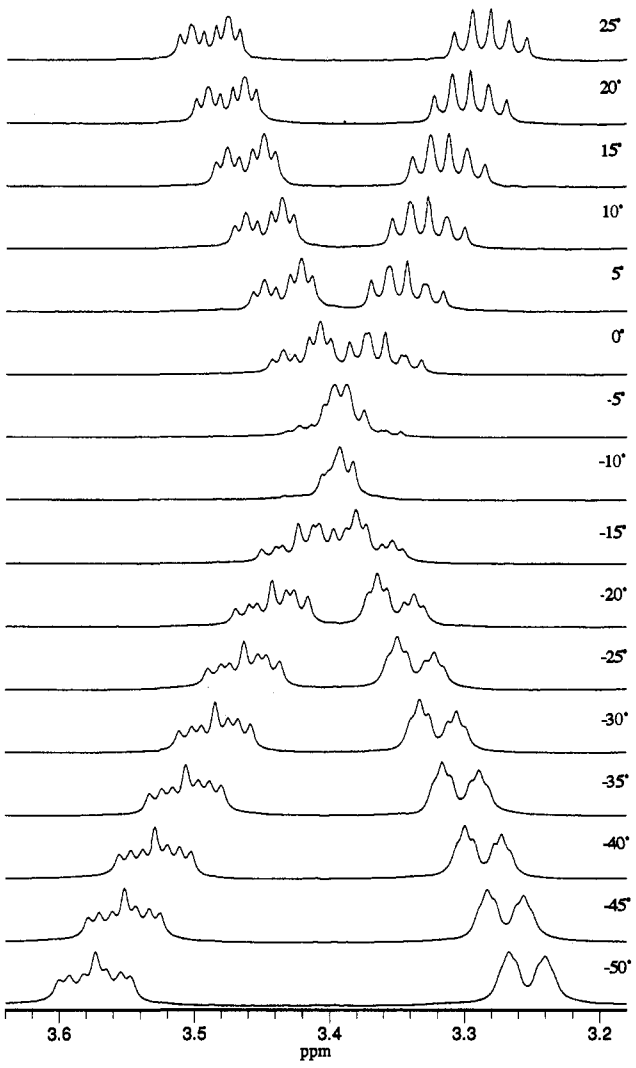


Figure 6. Variable-temperature ^{1}H NMR spectra of cryptophycin-3 (**3**) in $CDCl_3$ from 25 to −50 °C (5° intervals) showing the signals for the two protons on C-3 of unit C (C-7 in Figure 2). At 25 °C, δ 3.50 (3-$H_{pro\text{-}R}$, br ddd, $J = -13.6$, 5.0 and 3.7 Hz), 3.28 (3-$H_{pro\text{-}S}$,unresolved ddd, −13.6, 6.8, and 6.3 Hz); at −50 °C, δ 3.25 (3-$H_{pro\text{-}R}$, partially resolved dt, $J = -14$ and 2 Hz), 3.57 (3-$H_{pro\text{-}S}$, br ddd, −14, 9, and 3.5 Hz). Except for the unit C NH signal, which shifts from 6.93 ppm (br dd, $J = 6.8$ and 5.0 Hz) at 25 °C to 7.43 ppm (br dd, $J = 9$ and 2 Hz) at −50 °C, the chemical shifts and coupling constants for the remaining signals of **3** (not shown) are not changed significantly from 25 to −50 °C.

(*anti*) conformations shown in Figure 5c,d. Couplings of 6.3 and 3.7 Hz are now observed for $J_{2,3pro\text{-}S}$ and $J_{2,3pro\text{-}R}$, respectively, and the $J_{NH,3pro\text{-}S}$ and $J_{NH,3pro\text{-}R}$ couplings are 5−6.8 Hz. The J values represent average coupling constants for the two conformations which have comparable populations and are interconverting faster than the NMR time scale. As the temperature is lowered to −50 °C, the coupling constants gradually change to values that are similar to ones observed in DMSO solution, indicating that the equilibrium shifts in favor of the *syn* conformation (see Figure 6).

The preferred conformations of the side chains in DMSO solution appear to be identical with the ones shown in the crystal structure. For the 1-methyl-3-phenylprop-2(*E*)-enyl side chain of unit A, a dihedral angle of 180° exists between 5-H and 6-H in the crystal structure, but the 6.0−7.3 Hz coupling observed between these protons in solution, even at low temperatures, is smaller than expected. Nevertheless, strong NOE signals are detected between 3-H and 5-H, 5-H and 7-H, 4-$H_{pro\text{-}R}$ and 6-H, 6-H and 8-H, 7-H and 10-H (or 14-H), and 8-H and 14-H (or 10-H) in DMSO which are consistent with the crystal-state conformation.

For the (3-chloro-4-methoxyphenyl)methyl side chain in unit B, the $J_{2,3S}$ and $J_{2,3R}$ couplings are 11.6 and 3.5 Hz, entirely consistent with the dihedral angles of 180° and 60°, respectively, seen in the crystal structure. Significant NOE signals between NH and 3-H_S, NH and 5-H ($CDCl_3$ only), NH and 9-H ($CDCl_3$ only), 2-H and 5-H, and 2-H and 9-H indicate that the β-carbon in the side chain (C-4) is *anti* to the carbonyl carbon (C-1) rather than the amide nitrogen in the peptolide ring. Since the intensity of the NOE between 2-H and 5-H is much greater than the one between 2-H and 9-H, the preferred conformation of the aromatic ring is the one shown in the crystal structure where 2-H is *syn* to 5-H and *anti* to 9-H.

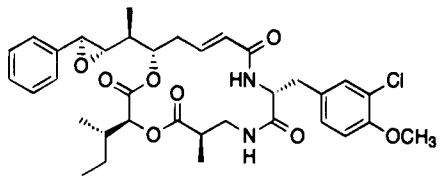
For the isobutyl side chain in unit D, the $J_{2,3R}$ and $J_{2,3S}$ couplings are 10.1−9.7 and 3.5−4.0 Hz, in agreement with dihedral angles of 180° and 60° seen in the crystal structure. Inspection of COSY spectra in various solvents reveal weak cross peaks between the 3-H_R and 4-H signals and intense cross peaks between the 3-H_S and 4-H signals, strongly suggesting small and large couplings for $J_{3R,4}$ and $J_{3S,4}$, respectively. In dichloromethane, $J_{3S,4}$ is estimated to be 11 Hz. Further evidence is provided by significant NOE signals that are observed between 2-H and 3-H_S and between 2-H and the protons of the *pro-S* methyl group that is *anti* to 3-H_R.

Similar coupling constants and NOE's are observed in the NMR spectra of most other cryptophycins, e.g., **1**, suggesting that the solution-state conformations of most cryptophycins are comparable with the one described here for **3**.

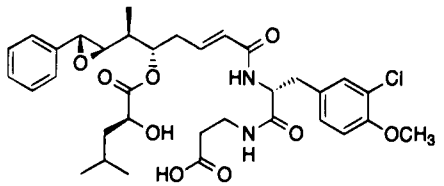
Chemical Stability. Pure cryptophycins appear to be relatively stable compounds. Contrary to results reported in a Merck patent,[1c] cryptophycin-1 (**1**) can be exposed to an organic solvent such as methanol or chloroform, or a mixture of an organic solvent and water, or an ionic, pH 7 buffered medium at room temperature for several weeks without appreciable decomposition due to solvolysis. The peptolide ring, which is 16-membered in all of the naturally-occurring cryptophycins except cryptophycin-26, is a stable to moderately strong acid; however, the epoxide functionality is very labile under these conditions. In a solution of sulfuric acid in 3:2 DME/H_2O, **1** has a half-life of approximately 4 h at pH 3, forming predominantly the *trans*-diol cryptophycin-15 (**40**) (Chart 3). In a solution of HCl in 3:2 DME/H_2O at pH 3, the *trans*-chlorohydrin cryptophycin-8 (**26**) is also formed. Similarly, the *trans*-bromohydrin cryptophycin-25 (**41**) is formed in a solution of HBr in DME. Exposure of **1** to a trace of acid (e.g., HCl) in methanol leads to methanolysis of the epoxide ring and the formation of cryptophycin-9 (**27**);[2] however, methanolysis of the ester bond connecting units C and D to give cryptophycin-5 (**5**) does not occur under these conditions.

The ester functionalities of the cryptophycins, on the other hand, are very susceptible to base hydrolysis, especially the one connecting units C and D. At pH 11.0 (potassium carbonate or methylamine in 3:2 DME/H_2O) the C−D ester bond of **1** is readily hydrolyzed at a $t_{1/2}$ of 50 min to form cryptophycin-13 (**30**) as the predominant product. A comparable rate of cleavage for the A−D ester bond in **30** requires a higher pH. At pH 11.5 (NaOH in 3:2 DME/H_2O), **30** is converted into cryptophycin-12 (**29**) and L-leucic acid at a $t_{1/2}$ of 54 min. In the formation of the tetrahydrofuran ring in **29**, hydrolysis of the A−D ester bond in **30** is followed by a rapid opening of the epoxide ring by the alcohol group that is generated in the A−B−C hydroxy acid intermediate. The C−D bond in cryptophycin-21 (**18**) is much more labile, hydrolyzing to hydroxy acid **42** at pH 11 at a $t_{1/2}$ of 15 min. The rate of

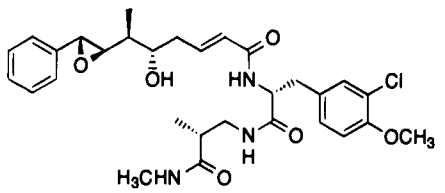
Chart 3



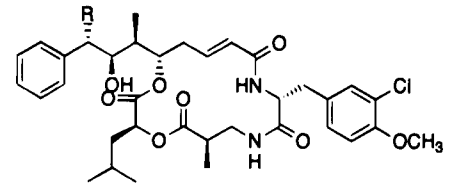
cryptophycin-101 **39**



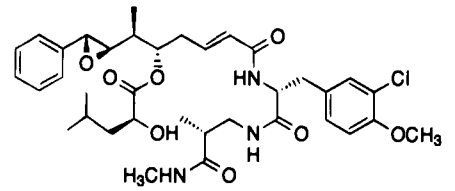
42



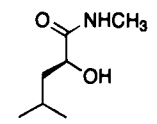
cryptophycin-66 **44**



cryptophycin-15 **40** R = OH
cryptophycin-25 **41** R = Br



cryptophycin-65 **43**



45

hydrolysis of the A−D ester bond in **42** appears to be comparable to that in **30**. Structural changes in the isobutyl side chain of unit D, however, affect the rate of hydrolysis of the A−D ester bond. Although the C−D ester bonds in cryptophycin-18 (**21**) and **3** are cleaved at comparable rates, the complete saponification of **21** requires more drastic conditions and longer reaction times compared with the complete saponification of **3**. In 0.03 N NaOH in 3:2 DME/H_2O, **3** is completely saponified to **31** in 2 h. In the same basic medium, **21** produces a 1:1 mixture of **31** and intermediate **38** after 8 h and requires over 24 h for complete saponification to **31**.

When **1** is treated with methylamine in DME, the C−D ester bond is selectively cleaved to give the acyclic *N*-methylamide cryptophycin-65 (**43**). The A−D ester bond in **43** is then cleaved to give an alcohol cryptophycin-66 (**44**) and the *N*-methylamide of L-leucic acid (**45**). Interestingly epoxide **43** is stable under these conditions and is not opened up by the alcohol group on C-6 of unit A to give a tetrahydrofuran.

Although pure cryptophycins are relatively stable in methanol solution, considerable decomposition of these peptolides occurs during the isolation procedure when methanol is used in the extraction and chromatography steps. Acyclic cryptophycin-5 (**5**) appears to be formed by a simple methanolysis of the C−D ester bond in **1**, although it is not clear why this degradation occurs so readily in the crude methanol extract and in the 3:1 methanol/water fractions obtained from reversed phase chromatography and HPLC. The origins of cryptophycin-6 (**6**) and -7 (**7**), which require hydrolyses of the A−D ester and the B−C amide bonds in **1**, are even less clear. Algal enzymes may be involved; however, further studies are needed.

Structure−Activity Relationships. Cytotoxicity IC_{50} values for **1** and six other previously described cryptophycins (**2**−**5**, **26**, **40**),[2] the 18 new analogs from the alga (**8**−**25**), and cryptophycin-25 (**41**) against three human tumor cell lines, viz. KB (a human nasopharyngeal carcinoma), LoVo (a human colorectal adenocarcinoma), and SKOV3 (a human ovarian carcinoma), are shown in Table 1. The data indicate that the intact 16-membered peptolide and *R,R*-epoxide rings, the chlorine and *O*-methyl groups in unit B, the methyl groups in units A and C, and the isobutyl group in unit D are needed for optimal cytotoxicity.

Table 1. Cytotoxicity Data for Cryptophycins

cryptophycin	KB IC_{50} (nM)	LoVo IC_{50} (nM)	SKOV3 IC_{50} (nM)
1 (**1**)	0.0092	0.010	0.020
2 (**2**)	0.073	0.110	0.057
3 (**3**)	3.13	1.88	4.36
4 (**4**)	16.5	21.5	34.5
5 (**5**)	10.9	8.75	10.8
8 (**26**)	0.019	0.0091	0.022
15 (**40**)	4.17	5.21	17.2
16 (**12**)	0.359	0.273	0.606
17 (**15**)	7.53	9.46	17.7
18 (**21**)	48.6	20.4	36.6
19 (**24**)	11.7	11.2	65.1
21 (**18**)	0.017	0.019	0.050
23 (**13**)	3.12	0.59	2.52
24 (**20**)	0.198	0.157	0.499
25 (**45**)	0.027	0.027	0.101
26 (**9**)	35.1	18.3	142
28 (**10**)	2.88	1.11	9.76
29 (**19**)	3.69	1.04	5.9
30 (**8**)	18.3	10.8	31.6
31 (**14**)	2.62	0.218	1.23
40 (**11**)	0.61	0.625	2.63
43 (**16**)	1.22	1.36	1.88
45 (**17**)	3.5	3.6	2.48
49 (**23**)	2.24	3.04	1.82
50 (**22**)	0.047	0.094	0.607
54 (**25**)	1.22	3.36	3.33

If the peptolide ring is opened, e.g., by methanolysis of the C−D ester bond (**5**, cryptophycin-5), a 1000-fold loss in cytotoxicity occurs. If the epoxide oxygen is eliminated (**3**, cryptophycin-3) or the epoxide ring hydrolyzed (**40**, cryptophycin-15), cytotoxicity is diminished a 100-fold. Interestingly, chlorohydrin **26** (cryptophycin-8) shows essentially the same *in vitro* activity as **1**, probably because **26** is a masked epoxide that can be converted into **1**. The corresponding bromohydrin (**41**, cryptophycin-25), however, is less cytotoxic (3- to 5-fold). Like **26**, **41** is probably converted into **1**; however, solvolysis of the benzylic bromide to the much less active diol may be competing with the formation of **1**. Removal of the methyl

Table 2. *In Vivo* Activity of Cryptophycin Analogs Administered Intravenously against Subcutaneous Transplanted Solid Tumors

expt no.	cryptophycin	SC tumor	no. of inj IV	total dosage (mg/kg)	% body wt loss at nadir	*T/C* (%)	gross log kill
1694	1 (**1**)	P03	8	16.0	gain	0	2.0
1560	1 (**1**)	C38	8	10.3	gain	6	1.5
1813	2 (**2**)	P03	10	37	−2	44	<1
2055	3 (**3**)	P03	11	59.6	0	28	<1
1843	3 (**3**)	P03	4	28.5	−9	54	<1
1769	5 (**5**)	C38	15	45	−2	>100	0
1825B	8 (**26**)	P03	11	106	−6	4	4.6[a]
1917	8 (**26**)	P03	12	60	−1	0	2.8
1954	8 (**26**)	C38	11	132	−9	0	>4.5[b]
1887B	8 (**26**)	C38	6	30	+2	0	3.0
2023	15 (**40**)	P03	5	60	−8	48	0
1878	16 (**12**)	P03	9	82	−1	89	0
1813	21 (**18**)	P03	9	27	−11 (1/5 dead)	61	0
2052	25 (**45**)	P03	6	18	−5	6	1.2

[a] Tumor infected; log kill may be excessively high. [b] 5/5 cures.

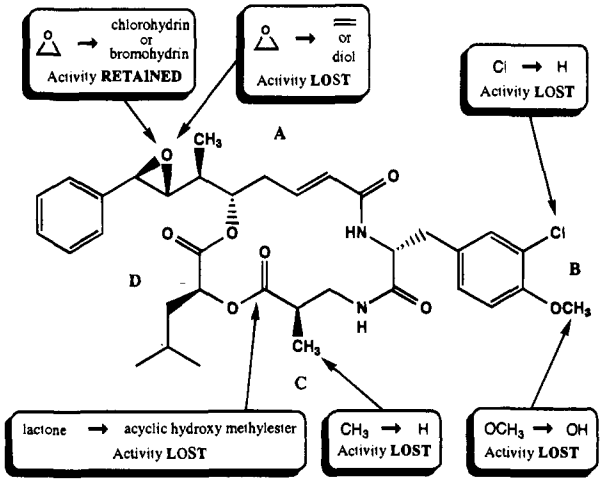


Figure 7. Summary of *in vivo* structure−activity relationship (SAR) studies of cryptophycin analogs.

group on C-6 of unit A (**11**, cryptophycin-40) produces a 50 to 100-fold loss of cytotoxicity, probably because the adjacent epoxide ring is more susceptible to hydrolysis, opening up to the much less active diol in the test medium. As expected, the corresponding styrene lacking the methyl group in unit A (**10**, cryptophycin-28) and **3** have comparable cytotoxicities. When the cytotoxicities of **3**, cryptophycin-30 (**8**), and cryptophycin-26 (**9**) are compared, it is evident that hydration of the Δ^2-double bond in unit A results in a 10-fold loss of activity and ring contraction to a 14-membered peptolide, e.g., **8** → **9**, results in a further (2-fold) loss of cytotoxicity.

Removal of the chlorine atom from unit B (**2**, cryptophycin-2) leads to a 10-fold reduction in cytotoxicity. If a second chlorine is introduced into unit B (**14**, cryptophycin-31) or the *O*-methyl group is eliminated from unit B (**12**, cryptophycin-16), the cytotoxicity is diminished 10- to a 100-fold. Loss of the methyl group on C-2 of unit C (**18**, cryptophycin-21), however, does not alter the cytotoxicity. Surprisingly, any change in the isobutyl group attached to C-2 of unit D, e.g., replacement by a *n*-propyl group (**22**, cryptophycin-50), an isopropyl group, or a *sec*-butyl group (**25**, cryptophycin-54), lessens the cytotoxicity substantially (10- to 100-fold).

Table 2 and Figure 7 summarize *in vivo* activity data for eight cryptophycin analogs against early stage murine pancreatic ductal adenocarcinoma P03 and/or colon adenocarcinoma C38 implanted subcutaneously in mice. Two of the analogs display activity. Cryptophycin-8 (**26**) was found to be more active than **1**, showing *T/C* values of 0−4% and gross log kill values of ≥2.8, values indicative of potential clinical activity.[27,28] Cures were obtained for one of the C38 trials for **26**. Cryptophycin-25 (**41**), however, showed *T/C* and gross log kill values that were comparable with those for **1**. This striking difference in activity suggests that the bromohydrin **41** is converted more rapidly into **1** *in vivo* than chlorohydrin **26**. This further suggests that **26** might have more time to accumulate at the tumor site before being transformed into **1**.

For five of the remaining six compounds, i.e., the cryptophycins missing the chlorine in unit B (**2**), the epoxide group (**3** and **40**), the intact macrolide ring (**5**), and the *O*-methyl group in unit B (**12**), the marginal to negative *in vivo* activities are consistent with the diminished cytotoxicities. The complete absence of *in vivo* activity for the cryptophycin missing the methyl group on C-2 of unit C (**18**), an analog that exhibits essentially the same cytotoxicity as **1**, however, can only be rationalized by the decomposition of this drug *in vivo* due to the increased susceptibility of the C−D ester bond to chemical cleavage.

Mechanism of Action. Preliminary studies[4] indicate that **1** irreversibly inhibits microtubule assembly, but whether **1** binds to a specific receptor on tubulin is not known. Evidence accumulated to date (e.g., comparison of cytotoxicity IC_{50}'s of over 50 analogs) suggests that the 1-(3-phenyloxiranyl)ethyl side chain[6] of **1** is involved in an irreversible, covalent binding to some receptor site. The initial binding of **1** to this unknown receptor is probably reversible and appears to involve the remainder of the molecule, i.e., the 1,4-dioxa-8,11-diazacyclohexadec-13(*E*)-ene-2,5,9,12-tetrone ring system with the (*S*)-2-methylpropyl and (*R*)-(3-chloro-4-methoxyphenyl)methyl side chains on C-3 and C-10 needed for optimal binding.

Apoptosis (programmed cell death) is rapidly induced in tumor cells exposed to **1** *in vitro*. Many of the characteristic features of apoptosis[29] are seen, including cell shrinkage and

(27) *T/C* values that are less than 42% are considered to be active by NCI standards; *T/C* values that are less than 10% are considered to have excellent activity and potential clinical activity by NCI standards. Gross log kill is defined as $T - C/3.2T_d$, where T is the median time in days for the tumors of the treated group of mice to reach 750 or 1000 mg, C is the median time in days for the tumors of the control group to reach 750 or 1000 mg, and T_d is the tumor volume doubling time. Gross log kill values of >2.8, 2.0−2.8, 1.3−1.9, 0.7−1.2, and <0.7 with duration of drug treatment of 5−20 days are scored ++++, +++, ++, +, and − (inactive), respectively. An activity rating of +++ to ++++, which is indicative of clinical activity, is needed to effect partial or complete regression of 100−300 mg sized masses of most transplanted solid tumors of mice.

(28) For a more detailed *in vivo* evaluation of cryptophycin-8 see: Corbett, T. H.; Valeriote, F. A.; Demchik, L.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Knight, J.; Jones, J.; Jones, L.; Foster, B.; Wiegand, R. A.; Lisow, L.; Golakoti, T.; Heltzel, C. E.; Ogino, J.; Patterson, G. M. L.; Moore, R. E. *J. Exp. Ther. Oncol.*, in press.

Table 3. Effect of Cryptophycin-1 (**1**) on Chromatin Condensation

concn (pM)	% chromatin condensation
0	0
50	15.9
100	21.3
200	34.7

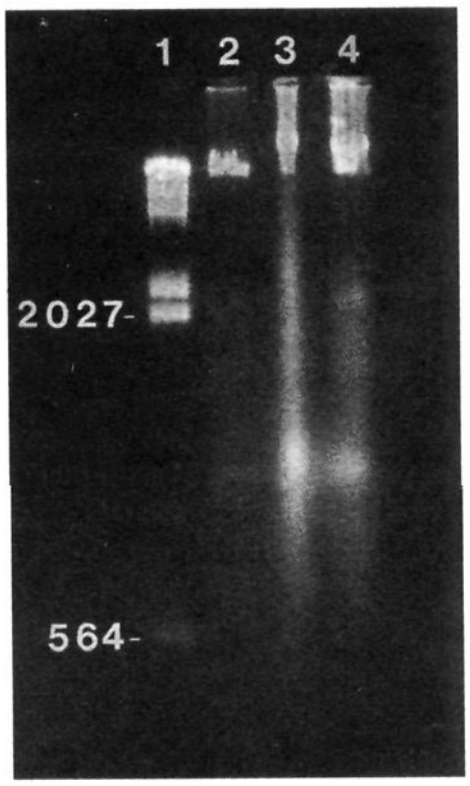


Figure 8. Cryptophycin-1 induced DNA fragmentation in L1210 cells. Lane 1 shows Lambda DNA/*Hind* III molecular weight markers. The remaining lanes show L1210 cells treated with vehicle (lane 2), 200 pM **1** (lane 3) and 1 μM dexamethazone as a positive control (lane 4) for 4 h at 37 °C.

loss of cytoplasm, membrane blebbing, and the formation of apoptotic bodies. Exposure of SKOV3 cells to picomolar doses of **1** caused significant nuclear chromatin condensation (Table 3), a morphological change consistent with apoptosis and attributable to DNA fragmentation, the latter of which could be visualized by agarose gel electrophoresis (Figure 8).

The ability of a cytotoxic agent to initiate apoptosis may play a critical role in cancer chemotherapy.[30] Cisplatin,[31] etoposide[32] and taxol[33] have all been shown to induce apoptosis, but much slower than **1**.

Experimental Section

Spectral and Computational Analyses. 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 chemical shifts are referenced in $CDCl_3$ to residual $CHCl_3$ (7.24 ppm) and in MeOH-d_4 to residual [CH_3-d_2] MeOD (3.30 ppm); ^{13}C chemical shifts are referenced in $CDCl_3$ and MeOH-d_4 to the solvent (77.0 and 49.5 ppm, respectively). Proton and carbon assignments are based on complete analysis of COSY, HMQC/HETCOR, HMBC/SINEPT, and NOE spectral data. Homonuclear ^{1}H connectivities were determined by a phase-sensitive, double-quantum filtered COSY experiment.[34] One-bond heteronuclear ^{1}H−^{13}C connectivities were determined by proton-detected HMQC[35] and carbon-detected HETCOR experiments. Two- and three-bond ^{1}H−^{13}C connectivities were determined by proton-detected HMBC[36] and carbon-detected selective INEPT experiments.[37] Homonuclear ^{1}H NOE's were obtained by difference NOE experiments using a 3s irradiation period and by two-dimensional NOESY and ROESY experiments.[38] UV spectra were measured in MeOH, unless otherwise noted. Optical rotations were measured in MeOH or $CHCl_3$ at 25 °C. Energy-minimized conformations were generated from CSC Chem3D Plus.

Cultivation of Alga: Hazards and Handling Procedures. All production cultures of *Nostoc* sp. strain GSV 224 were grown in 20 L glass carboys sparged with 5 L min^{-1} air containing 0.5% CO_2 as previously described.[2,39]

During the early stages of this investigation, workers handling cultures and extracts of the alga reported toxic reactions, with symptoms including inflammation, edema, and tenderness, especially around the eyes and nostrils. In most cases the symptoms passed in 4−5 days without treatment, although one extreme case required medical treatment with cortisone and Benadryl to control inflammation and edema.

Exposure to the toxic effects of the *Nostoc* cultures was limited by (1) reducing the production of aerosols during incubation and harvest and (2) using protective clothing. The aeration system was modified to vent exhaust sparge gas (after passage through a bacteria-retentive filter) through a chemical fume hood system. In order to limit handling and aerosol production during harvest of the algal biomass, a filtration system was constructed with commercially-available disposable polyester felt filter bags (14.2 × 81.2 cm) of 100 μm porosity enclosed in a filter housing. The culture suspension (typically 200 L) was transferred directly to this filtration apparatus from the culture vessels using a Teflon/polypropylene diaphragm pump. The filtrate was collected in closed-head drums until decontaminated by autoclaving for an extended period (the time required for destruction was determined by testing treated culture media for cytotoxicity and the presence of viable algae). The collected cell mass was frozen in the filter bag and then lyophilized. After lyophilization, the filter bag and dried algal mass (typically 100−150 grams per filter bag) were stored at −75 °C in sealed drums.

Personnel handling bulk cultures or harvested alga wore Tyvek protective coveralls, respirators equipped with HEPA filters, safety goggles, and two sets of gloves.

Since the institution of these safety practices, no toxic reactions have been observed among the workers.

Culture apparatus, equipment (e.g., freeze-dryer), and glassware used in the cultivation or subsequent extraction of *Nostoc* sp. GSV 224 has been found to retain and slowly release cytotoxic material, leading to cross-contamination of extracts of other algae currently under investigation in this laboratory. Our experience has shown that complete decontamination of glassware can be achieved by prolonged exposure (24 h) to 0.27 M NaOH in 9:1 EtOH/water or by heating in an oven to the annealing point of glass.

Isolation. Lyophilized *Nostoc* sp. GSV 224 (50 g) was removed from the filter bag[40] and extracted with 2 L of 1:5 CH_2Cl_2/MeCN for 48 h and the extract concentrated *in vacuo* to give a dark green solid (1 g). This solid was applied to an ODS-coated silica column (55 g, 7 × 5 cm) and subjected to flash chromatography with 1:3 MeCN/H_2O (0.8 L), 1:1 MeCN/H_2O (0.8 L), 65:35 MeCN/H_2O (1.0 L), MeOH (0.8 L), and CH_2Cl_2 (0.5 L). The material that was eluted with 65:35 MeCN/H_2O (420 mg) was further separated by reversed phase HPLC (Econosil C18, 10 μm, 25 cm × 22 mm, UV detection at 254 nm, 65:35 MeCN/H_2O, flow rate 6 mL/min) to give cryptophycin-1 (**1**, t_R 53.0 min, 220 mg) and a number of impure fractions. The fraction eluted from the Econosil C18 column at t_R 30.0 min was further purified

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by normal phase HPLC (Econosil silica 5-μm cartridge, 250 × 4.6 mm, 6:4 EtOAc/hexane, 3 mL/min) to give cryptophycin-16 (**12**, t_R 9.8 min, 3.0 mg). The fraction eluted from the Econosil C18 column at t_R 33.5 min was subjected to HPLC on the Econosil silica column using 55:45 EtOAc/hexane at 3 mL/min to give cryptophycin-24 (**20**, t_R 20.4 min, 0.8 mg). The fraction eluted from the Econosil C18 column at t_R 37.5 min was subjected twice to HPLC on the Econosil silica column, first using 1:1 EtOAc/hexane at 3 mL/min and second using 4:6 EtOAc/CH_2Cl_2 at 2.5 mL/min to give cryptophycin-23 (**13**, t_R 5.8 min, 1.2 mg) and cryptophycin-43 (**16**, t_R 12.8 min, 0.1 mg). The fraction eluted from the Econosil C18 column at t_R 41.8 min was subjected to HPLC on the Econosil silica column with 1:1 EtOAc/hexane at 3 mL/min to give cryptophycin-2 (**2**, t_R 12.0 min, 6 mg) and cryptophycin-21 (**18**, t_R 27.6 min, 14 mg) and a complex mixture of cryptophycins eluted at t_R 32.5 min. This latter fraction, accumulated from 400 g of dry alga, was chromatographed successively on a semipreparative column (Partisil C18, 250 × 9.4mm, 10 μm) with 65:35 MeCN/H_2O and a reversed phase analytical column (Econosil, 150 × 4.6mm, 5 μm) with 4:1:5 MeCN/MeOH/H_2O at 1.3 mL/min to give cryptophycin-50 (**22**, t_R 34.8, 0.4 mg) and cryptophycin-40 (**11**, t_R 38.8 min, 0.3 mg). The fraction eluted from the Econosil C18 column at t_R 47.0 min was subjected to HPLC on the Econosil silica column with 1:1 EtOAc/hexane at 3 mL/min to give cryptophycin-17 (**15**, t_R 12.8 min, 0.3 mg). Normal phase HPLC purification of the fraction eluted from the Econosil C18 column at t_R 59.0 as a shoulder to cryptophycin-1 yielded cryptophycin-45 (**17**, t_R 6.7 min, 0.1 mg), cryptophycin-26 (**9**, t_R 8.9 min, 0.5 mg), and cryptophycin-54 (**25**, t_R 19.8 min, 0.01 mg) on elution with 1:1 EtOAc/hexane. The fraction eluted from the Econosil C18 column as a broad peak (t_R 65−76 min) was subjected to HPLC on the Econosil silica column with 43:57 EtOAc/hexane at 2.5 mL/min to give cryptophycin-4 (**4**, t_R 19.6 min, 1.5 mg), cryptophycin-31 (**14**, t_R 9.4 min, 0.8 mg), cryptophycin-19 (**24**, t_R 25.8 min, 0.3 mg), cryptophycin-49 (**23**, t_R 28 min, 0.1 mg), cryptophycin-28 (**10**, t_R 29.0 min, 0.5 mg), impure cryptophycin-29 (**19**, t_R 52.5 min, 2.0 mg), and impure cryptophycin-30 (**8**, t_R 49 min, 3.0 mg). The latter two compounds were obtained pure after reversed phase HPLC (Econosil C18, 10 μm, 250 × 10 mm, 3:1 MeOH/H_2O). The fraction eluted from the Econosil C18 column at t_R 89.8 min was subjected to HPLC on the Econosil silica column with 1:1 EtOAc/hexane to give cryptophycin-3 (**3**, t_R 16.4 min, 3.0 mg). The fraction eluted from the Econosil C18 column at t_R 94.6 min was subjected to HPLC on the Econosil silica column with 45:55 EtOAc/hexane at 3 mL/min to give cryptophycin-18 (**21**, t_R 19.2, 0.8 mg).

Cryptophycin-30 (8): $[\alpha]_D$ −12.3° ($CHCl_3$, *c* 1.53); UV λ_{max} (ϵ) 204 (51 100), 232 (15 500), 252 (17 300), 280 (3200); IR (neat) ν_{max} 3414, 3306, 2961, 1738, 1729, 1660, 1504, 1258, 1205, 1183, 1066, 695 cm^{-1}; EIMS *m/z* (rel intensity) 656/658 (1.0/0.3), 638/640 (3.0/1.0), 525/527 (3.8/1.3), 412/414 (10.5/3.6), 280/282(10.3/3.8), 227 (29), 195/197 (48/17), 155/157 (74/21), 131 (100); high resolution EIMS *m/z* 656.2852 (calcd for $C_{35}H_{45}ClN_2O_8$, 1.2 mmu error); ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 2.25 (2, dd; −16.0 and 9.6), 2.64 (2, brd; 16.0), 3.89 (3, m), 2.51 (3-OH, d; 6.4), 1.77 (4, ddd; −14.3, 9.8 and 2.1), 1.88 (4, ddd; −14.3, 11.3 and 3.8), 4.88 (5, ddd; 11.3, 6.2 and 2.1), 2.48−2.58 (6, m), 1.10 (6-Me, d; 6.8), 5.99 (7, dd; 15.9 and 9.0), 6.40 (8, d; 15.9), 7.28−7.33 (10/11/13/14, m), 7.23 (12, m); B 4.58−4.62 (2, m), 6.61 (2-NH, d; 8.1), 3.09 (3, dd; −14.2 and 5.6), 3.15 (3, dd; −14.2 and 7.3), 7.22 (5, d; 2.1), 3.86 (7-OMe, s), 6.83 (8, d; 8.3), 7.07 (9, dd; 8.3 and 2.1); C 2.64−2.70 (2, m), 1.21 (2-Me, d; 7.3), 3.26 (3, ddd; −13.6, 7.3 and 6.3), 3.63 (3, ddd; −13.6, 6.3 and 3.9), 6.75 (3-NH, br t; 6.3); D 4.83 (2, dd; 9.6 and 4.1), 1.39−1.45 (3, m), 1.61−1.70 (3/4, m). 0.79 (4-Me, d; 6.4), 0.76 (5, d; 6.4); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 171.6 (1), 42.4 (2), 66.0 (3), 41.3 (4), 76.0 (5), 42.0 (6), 17.3 (6-Me), 130.0 (7), 131.9 (8), 136.7 (9), 126.1 (10/14), 128.6 (11/13), 127.6 (12); B 170.8 (1), 54.3 (2), 35.1 (3), 130.1 (4), 131.1 (5), 122.2 (6), 153.9 (7), 56.1 (7-OMe), 112.1 (8), 128.7 (9); C 175.6 (1), 39.7 (2), 13.8 (2-Me), 41.5 (3), *D* 171.9 (1), 72.1 (2), 39.1 (3), 24.6 (4), 21.4 (4-Me), 22.7 (5).

Cryptophycin-26 (9): $[\alpha]_D$ +28.2° ($CHCl_3$, *c* 1.31); UV λ_{max} (ϵ) 206 (40 200), 232 (13 700), 252 (16 400), 282 (3540); IR (neat) ν_{max} 3299, 2960, 1732, 1644, 1504, 1258, 1209 cm^{-1}; EIMS *m/z* (rel intensity) 656/658 (0.5/0.1, M^+), 638/640 (1.7/1.0), 525/527 (3.7/1.8), 412/414 (10/4), 280/282 (12/11), 227 (20), 195 (48), 131 (68); high-resolution EIMS *m/z* 656.2836 (calcd for $C_{35}H_{45}ClN_2O_8$, 2.8 mmu error), 638.2712 (calcd for $C_{35}H_{43}ClN_2O_7$, 4.7 mmu error); ^1H NMR data, see Table 4; ^{13}C NMR data, see Table 5.

Cryptophycin-28 (10): $[\alpha]_D$ +65.6° (MeOH, *c* 0.93); UV λ_{max} (ϵ) 204 (48 000), 230 (19 300), 248 (18 700), 280 (3400); IR (neat) ν_{max} 3413, 3270, 2958, 1745, 1726, 1665, 1504, 1258, 1197, 1175, 1066, 694 cm^{-1}; EIMS *m/z* (rel intensity) 624/626 (3.0/1.3), 412/414 (70/24), 280/282(13/6), 213 (100), 195/197 (86/40); high-resolution EIMS *m/z* 624.2626 (calcd for $C_{34}H_{41}ClN_2O_7$, −2.4 mmu error); ^1H NMR data: see Table 4, ^{13}C NMR data, see Table 5.

Cryptophycin-40 (11): $[\alpha]_D$ +41.6° ($CHCl_3$, *c* 0.31); UV ($CHCl_3$) λ_{max} (ϵ) 242 (4974), 266 (3911), 274 (3666), 286 (2359), 328 (511); IR (neat) ν_{max} 3415, 2959, 1748, 1723, 1667, 1505, 1463, 1289, 1176 cm^{-1}; EIMS *m/z* (rel intensity) 640/642 (5/2), 280/282 (7/3), 213 (13), 195/197 (51/17), 155 (29), 141 (32), 121 (28), 91 (100), 69 (47); high-resolution EIMS *m/z* 640.2570 (calcd for $C_{34}H_{41}ClN_2O_8$, −1.9 mmu error); ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.77 (2, d; 15.1), 6.72 (3, ddd; 15.1, 9.7 and 4.9), 2.43 (4, m), 2.58 (4, m), 5.33 (5, m), 1.89 (6, ddd; −12.9, 8.9 and 5.0), 2.13 (6, ddd; −12.9, 9.3 and 4.8), 2.98 (7, ddd; 8.9, 4.8 and 1.9), 3.64 (8, d; 1.9), 7.31−7.39 (10/11/13/14, m), 7.22 (12, m); B 4.82 (2, m), 5.64 (2-NH, d; 8.6), 3.04 (3, dd; −14.3 and 7.5), 3.14 (3, dd; −14.3 and 5.4), 7.21 (5, d; 2.0), 3.87 (7-OMe, s), 6.84 (8, d; 8.3), 7.08 (9, dd; 8.3 and 2.0); C 2.72 (2, m), 1.23 (2-Me, d; 7.3), 3.31 (3, dt; −13.7 and 6.0), 3.49 (3, ddd; −13.7, 6.0 and 3.9), 6.96 (3-NH, br t; 6.0); D 4.85 (2, dd; 6.7 and 3.4), 1.42 (3, m), 1.64−1.75 (3/4, m), 0.86 (4-Me, d, 6.7), 0.87 (5, d, 6.7); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.3 (1), 125.2 (2), 140.9 (3), 39.0 (4), 72.0 (5), 37.7 (6), 59.0 (7), 58.7 (8), 140.9 (9), 125.6 (10/14), 128.7 (11/13), 128.5 (12); B 170.9 (1), 53.6 (2), 35.1(3), 129.8 (4), 131.0 (5), 122.5 (6), 157.0 (7), 56.1 (7-OMe), 112.3 (8), 128.4 (9); C 175.6 (1), 38.3 (2), 14.1 (2-Me), 41.1 (3); D 170.9 (1), 71.4 (2), 39.4 (3), 24.5 (4), 21.5 (4-Me), 22.8 (5).

Cryptophycin-16 (12): $[\alpha]_D$ +41.3° (MeOH, *c* 5.2); UV λ_{max} (ϵ) 206 (38 300), 218 (28 200), 248 (2950), 282 (1820); IR (neat) ν_{max} 3402, 3270, 2960, 1748, 1724, 1676, 1514, 1466, 1343, 1239, 1177 cm^{-1}; EIMS *m/z* (rel intensity) 640/642 (66/27), 398/400 (47/16), 265 (55), 227 (93), 181 (100); high-resolution EIMS *m/z* 640.2568 (calcd for $C_{34}H_{41}ClN_2O_8$, −1.6 mmu error); ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.74 (2, d; 15.7), 6.67 (3, ddd; 15.7, 10.1 and 5.4), 2.45 (4, ddd; −14.3, 10.8 and 10.1), 2.55 (4, br dd; −14.3 and 5.4), 5.15 (5, ddd; 10.8, 4.8 and 1.8), 1.77−1.83 (6, m), 1.14 (6-Me, d; 7.0), 2.92 (7, dd; 7.5 and 2.0), 3.69 (8, d; 2.0), 7.24−7.26 (10/14, m), 7.33−7.39 (11/12/13, m); B 4.78−4.82 (2, m), 5.64 (2-NH, d; 8.8), 3.03 (3, dd; −14.5 and 7.0), 3.11 (3, dd; −14.5 and 5.6), 7.17 (5, d; 2.2), 5.61(7-OH, s), 6.91 (8, d; 8.3), 7.00 (9, dd; 8.3 and 2.2); C 2.67−2.74 (2, m), 1.22 (2-Me, d; 7.3), 3.28 (3, dt; −13.6 and 6.0), 3.49 (3, ddd; −13.6, 6.0 and 4.1), 6.92 (3-NH, br t; 6.0); D 4.83 (2, dd; 10.1 and 3.3), 1.33−1.39 (3, m), 1.67−1.75 (3, m), 1.67−1.75 (4, m), 0.85 (4-Me, d; 6.8), 0.86 (5, d; 6; ^{13}C NMR ($CDCl_3$) unit δ (carbon position) 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), 58.9 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.6 (12); B 170.9 (1), 53.6 (2), 35.1 (3), 129.9 (4), 129.6 (5), 120.0 (6), 150.4 (7), 116.4 (8), 129.2 (9); C 175.5 (1), 38.3 (2), 14.1 (2-Me), 41.2 (3); D 170.8 (1), 71.3 (2), 39.4 (3), 24.6 (4), 21.3 (4-Me), 22.9 (5).

Cryptophycin-23 (13): $[\alpha]_D$ +47° (MeOH, *c* 1.55); UV λ_{max} (ϵ) 208 (44 200), 252 (3530), 290 (1860); IR (neat) ν_{max} 3284, 2960, 1747, 1724, 1653, 1540, 1490, 1339, 1272, 1174 cm^{-1}; EIMS *m/z* (rel intensity) 674/675/678 (47/35/8), 432/434/436 (11/5/2), 299/301/303 (39/30/7), 227 (64), 215/217/219 (31/20/8), 141 (100); high-resolution EIMS *m/z* 674.2164 (calcd for $C_{34}H_{40}Cl_2N_2O_8$, −0.3 mmu error). ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.77 (2, d; 15.4), 6.65 (3, ddd; 15.4, 9.8 and 5.8), 2.47 (4, ddd; −14.2, 10.6 and 9.8), 2.55 (4, br dd; −14.2 and 5.8), 5.13 (5, ddd; 10.6, 4.6 and 1.6), 1.81 (6, m), 1.15 (6-Me, d; 6.9), 2.93 (7, dd; 7.6 and 2.0), 3.70 (8, d; 2.0), 7.22-7.26 (10/14, m), 7.32−7.39 (11/12/13, m); B 4.79−4.83 (2, m), 5.69 (2-NH, d; 8.6), 3.00 (3, dd; −14.4 and 7.0), 3.11 (3, dd; −14.4 and 5.6), 7.13 (5/9, s), 5.78 (7-OH, s); C 2.69−2.77 (2, m), 1.22 (2-Me, d; 7.1), 3.19 (3, dt; −13.5 and 6.2), 3.58 (3, ddd; −13.5, 6.2 and 4.1), 6.82 (3-NH, br t; 6.2); D

Table 4. ^{1}H NMR Data for Cryptophycins-18, -19, -21, -26, -28, and -50[a]

position	18 (21)	19 (24)	21 (18)	26 (9)	28 (10)	50 (22)
A 2	5.76 (d, 15.5)	5.76 (d, 15.3)	5.73 (d,15.2)	2.46 (dd, −14.8, 7.8)	5.78 (d, 15.6)	5.73 (d, 15.7)
2′				2.58 (dd, −14.8, 3.0)		
3	6.65 (ddd, 15.5, 9.2, 6.2)	6.64 (ddd, 15.3, 9.1, 6.2)	6.68 (ddd, 15.2, 9.9, 4.9)	5.44−5.48 (m)	6.71 (ddd, 15.6, 9.9, 5.4)	6.67 (ddd, 15.7, 9.7, 5.4)
4	2.38−2.47 (m)	2.36−2.41 (m)	2.40−2.48 (m)	1.86−1.90 (m)	2.37−2.44 (m)	2.40−2.48 (m)
4′	2.38−2.47 (m)	2.44−2.49 (m)	2.50−2.60 (m)		2.47−2.58 (m)	2.53−2.58 (m)
5	5.08 (ddd, 10.6, 4.9, 2.2)	5.04 (ddd, 10.8, 5.2, 1.8)	5.19 (ddd, 11.2, 5.1, 1.5)	3.61 (td, 6.8, 3.9)	5.14−5.21 (m)	5.13 (ddd, 11.2, 5.0. 1.7)
6	2.53−2.62 (m)	2.53−2.61 (m)	1.76−1.84 (m)	2.32−2.41 (m)	2.47−2.58 (m)	1.76−1.81 (m)
6-Me	1.15 (d, 6.8)	1.15 (d, 6.9)	1.14 (d, 7.1)	1.14 (d, 6.8)		1.15 (d, 6.9)
7	6.07 (dd, 15.9, 8.5)	6.05 (dd, 15.8, 8.5)	2.92 (dd, 7.5, 2.0)	6.06 (dd, 16.0, 8.7)	6.07 (dt, 15.8, 7.4)	2.91 (dd, 7.5, 1.9)
8	6.43 (d, 15.9)	6.43 (d, 15.8)	3.69 (d, 2.0)	6.47 (d, 16.0)	6.44 (d, 15.8)	3.69 (d, 1.9)
10/14	7.29−7.35 (m)	7.29−7.35 (m)	7.23−7.26 (m)	7.37 (br d, 7.8)	7.27−7.38 (m)	7.24−2.26 (m)
11/13	7.29−7.35 (m)	7.29−7.35 (m)	7.33−7.38 (m)	7.32 (br t, 7.8)	7.27−7.38 (m)	7.33−7.38 (m)
12	7.23 (dd, 7.1, 1.6)	7.23 (dd, 6.9, 1.7)	7.33−7.38 (m)	7.22−7.28 (m)	7.20−7.24 (m)	7.33−7.38 (m)
B 2	4.80−4.85 (m)	4.81−4.86 (m)	4.74 (ddd, 8.4, 7.2, 5.9)	4.73 (ddd, 8.6, 8.0, 6.3)	4.79−4.85 (m)	4.81 (ddd, 8.3, 7.2, 5.5)
2-NH	5.65 (d, 8.7)	5.67 (d, 8.9)	5.68 (d, 8.4)	6.14 (d, 8.6)	5.67 (d, 8.5)	5.61 (d, 8.3)
3	3.04 (dd, −14.5, 7.1)	3.04 (dd, −14.3, 7.1)	2.98 (dd, −14.3, 7.2)	2.84 (dd, −14.4, 8.0)	3.04 (dd, −14.5, 7.2)	3.03 (dd, −14.4,7.2)
3′	3.14 (dd, −14.5, 5.5)	3.14 (dd, −14.3, 5.3)	3.14 (dd, −14.3, 5.9)	3.18 (dd, −14.4, 6.3)	3.14 (dd, −14.5, 5.4)	3.13 (dd, −14.4,5.5)
5	7.21 (d, 2.3)	7.22 (d, 2.0)	7.21 (d, 2.0)	7.21 (d, 2.2)	7.22 (d, 2.0)	7.21 (d, 2.0)
7-OMe	3.86 (s)	3.86 (s)	3.86 (s)	3.85 (s)	3.87 (s)	3.87 (s)
8	6.83 (d, 8.3)	6.83 (d, 8.2)	6.83 (d, 8.4)	6.82 (d, 8.6)	6.84 (d, 8.5)	6.83 (d, 8.4)
9	7.08 (dd, 8.3, 2.3)	7.08 (dd, 8.2, 2.0)	7.07 (dd, 8.4, 2.0)	7.08 (dd, 8.6, 2.2)	7.08 (dd, 8.5, 2.0)	7.07 (dd, 8.4, 2.0)
C 2	2.69−2.77 (m)	2.72−2.78 (m)	2.50−2.60 (m)	2.84−2.90 (m)	2.68−2.74 (m)	2.68−2.74 (m)
2-Me	1.23 (d, 7.2)	1.23 (d, 7.1)		1.19 (d, 7.0)	1.21 (d, 7.2)	1.22 (d, 7.3)
3	3.23 (dt, −13.5, 7.0)	3.16−3.23 (m)	3.48−3.56 (m)	3.01 (ddd, −13.4, 10.6, 5.1)	3.29 (dt, −13.5, 7.0)	3.29 (dt, −13.6, 6.7)
3′	3.56 (ddd, −13.5, 6.0, 4.0)	3.56−3.62 (m)	3.42−3.48 (m)	3.73 (ddd, −13.4, 7.8, 4.7)	3.49 (ddd, −13.5, 4.9, 3.8)	3.49 (ddd, −13.6, 6.7, 5.0)
3-NH	6.85 (dd, 7.0, 6.0)	6.80 (brt, 6.7)	6.90 (br t, 5.8)	6.72 (dd, 7.8,5.1)	6.95 (dd, 7.0, 4.9)	6.92 (brt, 6.7)
D 2	4.80 (d, 4.6)	4.73 (d, 4.2)	4.89 (dd, 10.0, 3.3)	4.95 (dd, 9.7, 4.2)	4.79−4.85 (m)	4.75 (dd, 9.2, 3.7)
3	1.86−1.89 (m)	2.07−2.11 (m)	1.62−1.72 (m)	1.62−1.72 (m)	1.57−1.67 (m)	1.49−1.59 (m)
3′			1.28−1.34 (m)	1.79−1.84 (m)	1.37−1.43 (m)	1.62−1.70 (m)
3-Me	0.94 (d, 7.0)	0.95 (d, 6.9)				
4	1.20−1.26 (m)	0.84 (d, 6.9)	1.62−1.72 (m)	1.62−1.72 (m)	1.57−1.67 (m)	1.27−1.38 (m)
4′	1.39−1.44 (m)					1.27−1.38 (m)
4-Me			0.83 (d, 6.4)	0.90 (d, 6.4)	0.74 (d, 6.3)	
5	0.77 (t, 7.4)		0.84 (d, 6.4)	0.95 (d, 6.4)	0.76 (d, 6.3)	0.84 (t, 7.3)

[a] Spectra taken in $CDCl_3$, at 500 MHz; chemical shifts from TMS (multiplicity, *J* in Hz).

4.84 (2, dd; 9.9 and 3.2), 1.35−1.41 (3, m), 1.68−1.75 (3, m), 1.68−1.75 (4, m), 0.86 (4-Me, d; 6.7), 0.87 (5, d; 6.7); ^{13}C NMR ($CDCl_3$) *unit* δ (carbon position) A 165.4 (1), 125.4 (2), 140.9 (3), 36.7 (4), 76.3 (5), 40.6 (6), 13.5 (6-Me), 63.0 (7), 58.9 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.6 (12); B 170.7 (1), 53.3 (2), 35.0 (3), 130.3 (4), 129.0 (5/9), 121.0 (6/8), 146.7 (7); C 175.3 (1), 38.4 (2), 13.9 (2-Me), 41.5 (3); D 170.8 (1), 71.3 (2), 39.4 (3), 24.6 (4), 21.3 (4-Me), 22.9 (5).

Cryptophycin-31 (14): $[\alpha]_D$ +50.6° (MeOH, *c* 1.13); UV λ_{max} (ϵ) 206 (45 500), 220 (28 300), 280 (600); IR (neat) ν_{max} 3412, 3272, 2961, 1745, 1725, 1678, 1537, 1481, 1270, 1196, 1176, 1000, 698 cm^{-1}; EIMS *m/z* (rel intensity) 688/690/692 (1.2/1.0/0.4), 446/448/450 (7.9/6.7/3.1), 314/316/318 (17/11/3), 91 (100); high resolution EIMS *m/z* 688.2336 (calcd for $C_{35}H_{42}Cl_2N_2O_8$, −1.8 mmu error). ^{1}H-NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.78 (2, d; 15.5), 6.66 (3, ddd; 15.5, 9.4 and 6.0), 2.47 (4, ddd; −14.1, 10.8 and 9.4), 2.56 (4, br dd; −14.1 and 6.0), 5.14 (5, ddd; 10.8, 4.7 and 1.7), 1.78-1.85 (6, m), 1.15 (6-Me, d; 7.1), 2.93 (7, dd; 7.5 and 1.9), 3.70 (8, d; 1.9), 7.24−7.26 (10/14, m), 7.34−7.39 (11/12/13, m); B 4.80−4.86 (2, m), 5.68 (2-NH, d; 9.0), 3.00 (3, dd; −14.4 and 7.3), 3.14 (3, dd; −14.4 and 5.6), 7.16 (5/9, s), 3.87 (7-OMe, s); C 2.74 (2, m), 1.22 (2-Me, d; 7.1), 3.20 (3, m), 3.58 (3, ddd; −13.5, 5.6 and 4.1), 6.82 (3-NH, br t; 5.6); D 4.80−4.86 (2, m), 1.38 (3, m), 1.66−1.75 (3/4, m). 0.87 (4-Me, d; 6.8), 0.86 (5, d; 6.8); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.4 (1), 125.4 (2), 141.0 (3), 36.7 (4), 76.3 (5), 40.6 (6), 13.5 (6-Me), 63.0 (7), 58.9 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.6 (12); B 170.8 (1), 53.3 (2), 35.2 (3), 134.5 (4), 129.6 (5/9), 129.3 (6/8), 151.2 (7), 60.6 (7-OMe); C 175.3 (1), 38.3 (2), 13.9 (2-Me), 41.5 (3), *D* 170.6 (1), 71.3 (2), 39.4 (3), 24.6 (4), 22.9 (4-Me), 21.3 (5).

Cryptophycin-17 (15): $[\alpha]_D$ +27.8° ($CHCl_3$, *c* 0.37); UV λ_{max} (ϵ) 208 (55 100), 232 (21 700), 248 (21 800), 282 (3200); IR (neat) ν_{max} 3412, 2958, 1750, 1723, 1668, 1504, 1463, 1290, 1177, 751 cm^{-1}; EIMS *m/z* (relative intensity) 624/626 (5/2), 483/485 (4/1), 398/400 (100/35), 379 (15), 149 (50); high-resolution EIMS *m/z* 624.2616 (calcd for $C_{34}H_{41}ClN_2O_7$, −1.4 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon positions, multiplicities; *J* in Hz) A 5.78 (2, dd; 15.3 and 0.9), 6.68 (3, ddd; 15.3, 9.8 and 5.6), 2.37 (4, ddd; −14.5, 11.1 and 9.8), 2.52 (4, br dd; −14.5 and 5.6), 4.99 (5, ddd; 11.1, 6.5 and 1.9), 2.56 (6, m), 1.13 (6-Me, d; 6.9), 6.01 (7, dd; 16.0 and 8.9), 6.41 (8, d; 16.0), 7.28−7.34 (10/11/13/14, m), 7.22 (12, m); B 4.78−4.83 (2, m), 5.79 (2-NH, d; 8.5), 3.12 (3, dd; −14.5 and 7.1), 3.02 (3, dd; −14.5 and 5.6), 7.18 (5, d; 2.0), 5.87 (7-OH, br s), 6.90 (8, d; 8.4), 7.0 (9, dd; 8.4 and 2.0); C 2.68−2.74 (2, m), 1.21 (2-Me, d; 7.3), 3.26 (3, dt; −13.6 and 6.0), 3.51 (3, ddd; −13.6, 6.0 and 4.0), 6.95 (3-NH, br t, 6.0); D 4.84 (2, dd; 9.8 and 3.3), 1.60−170 (3, m), 1.33−1.39 (3, m), 1.60−1.70 (4, m), 0.77 (4-Me, d; 6.4), 0.72 (5, d; 6.4); ^{13}C NMR ($CDCl_3$) unit δ (carbon positions) A 165.6 (1), 125.2 (2), 141.4 (3), 36.4 (4), 77.5 (5), 42.2 (6), 17.3 (6-Me), 130.1 (7), 131.8 (8), 136.7 (9), 126.1 (10/14), 128.6 (11/13), 127.6 (12); B 171.0 (1), 53.6 (2), 35.1 (3), 129.9 (4), 129.2 (5), 119.9 (6), 150.4 (7), 116.4 (8), 129.7 (9); C 175.5 (1), 38.3 (2), 14.0 (2-Me), 41.2 (3); D 170.9 (1), 71.6 (2), 39.5 (3), 24.5 (4), 21.2 (4-Me), 22.7 (5).

Cryptophycin-43 (16): $[\alpha]_D$ +20° ($CHCl_3$, *c* 0.2); UV ($CHCl_3$) λ_{max} (ϵ) 250 (20512), 282 (4083), 294 (1734); IR (neat) ν_{max} 3400, 3272, 2927, 1727, 1660, 1516, 1455, 1242, 1175 cm^{-1}; EIMS *m/z* (rel intensity) 533 (24), 484 (3), 445 (14), 398 (9), 364 (29), 227 (59), 149 (67), 91 (100); high-resolution EIMS *m/z* 590.3044 (calcd for $C_{34}H_{42}N_2O_7$, −5.2 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.75 (2, d; 15.3), 6.69 (3, ddd; 15.3, 10.1 and 5.3), 2.37 (4, ddd; −14.2, 10.8 and 10.1), 2.52 (4, m), 5.01 (5, ddd; 10.8, 6.4 and 1.8), 2.55 (6, m), 1.13 (6-Me, d; 6.9),

Table 5. ^{13}C NMR Data for Cryptophycins −18, −19, −26, −28, and −50[a]

C	18 (**21**)	19 (**24**)	21 (**18**)	26 (**9**)	28 (**10**)	50 (**22**)
A 1	165.5	165.5	165.5	170.0	165.4	165.3
2	125.2	125.3	125.3	41.5	125.2	125.3
3	141.5	141.3	141.0	71.4	141.2	141.0
4	36.4	36.3	36.7	37.3	38.5	36.9
5	77.7	77.7	75.9	71.9*	73.5	76.3
6	41.9	42.0	40.6	43.6	38.6	40.8
6-Me	17.1	17.1	13.5	16.6		13.6
7	129.8	129.9	63.0	130.8	124.1	63.2
8	131.9	131.9	59.0	132.5	133.8	59.1
9	136.8	136.8	136.7	136.8	136.7	136.8
10/14	126.2	126.1	125.6	126.2	126.1	125.5
11/13	128.6	128.6	128.7	128.6	128.6	128.7
12	127.6	127.6	128.5	127.6	127.6	128.5
B 1	171.0	171.0	170.7	170.9	170.9	170.9
2	53.5	53.4	53.9	53.2	53.6	53.6
3	35.1	35.1	35.0	34.7	35.1	35.1
4	129.9	130.0	129.8	130.3	129.8	129.8
5	131.1	131.1	130.9	131.1	131.0	131.0
6	122.4	122.4	122.4	122.2	122.4	122.5
7	153.9	153.9	153.9	153.8	154.0	154.0
7-OMe	56.1	56.1	56.1	56.1	56.1	56.1
8	112.2	112.2	112.2	112.2	112.3	112.3
9	128.5	128.5	128.3	128.5	128.4	128.5
C 1	175.3	175.1	172.6	174.3	175.6	175.6
2	38.6	38.7	32.4	40.1	38.3	38.4
2-Me	14.0	13.9		14.4	14.0	14.1
3	41.4	41.5	34.4	42.5	41.2	41.2
D 1	169.5	169.6	170.5	170.7	170.9	170.4
2	76.6	76.9	71.2	71.8*	71.6	72.4
3	36.2	29.8	39.5	38.9	39.6	32.7
3-Me	15.5	19.0				
4	24.2	16.7	24.4	24.6	24.5	18.4
4-Me			21.2	21.6	22.6	
5	11.2		22.8	22.9	21.5	13.5

[a] Spectra taken in $CDCl_3$, at 125 MHz; chemical shifts in ppm from TMS. *Interchangeable.

6.01 (7, dd; 15.8 and 8.9), 6.41 (8, d; 15.8), 7.21−7.34 (10/11/12/13/14, m); B 4.80 (2, m), 5.64 (2-NH, d; 8.4), 3.06(3, dd; −14.5 and 7.2), 3.13 (3, dd; −14.5 and 5.3), 7.06 (5/9, d; 8.4), 6.74 (6/8, d; 8.4); C 2.69 (2, m), 1.22 (2-Me, d; 7.3), 3.33 (3, m), 3.44 (3, dt; −14.0 and 4.7), 7.00 (3-NH, m); D 4.84 (2, dd; 10.0 and 3.6), 1.60−1.67 (3, m), 1.35 (3, m), 1.60−1.67 (4, m), 0.73 (4-Me, d; 6.7), 0.76 (5, d; 6.4); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A nd (1), 125.2 (2), 141.5 (3), 36.5 (4), 77.5 (5), 42.3 (6), 17.3 (6-Me), 130.1 (7), 131.8 (8), 136.8 (9), 126.2 (10/14), 128.6 (11/13), 127.6 (12); B nd (1), 53.8 (2), 35.3 (3), 129.8 (4), 130.5 (5/9), 115.6 (6/8), 154.6 (7); C nd (1), 38.3 (2), 14.1 (2-Me), 41.0 (3); D nd (1), 71.6 (2), 39.6 (3), 24.5 (4), 22.9 (4-Me), 21.2 (5).

Cryptophycin-45 (17): $[\alpha]_D$ +72.0° (MeOH, *c* 0.122); UV ($CHCl_3$) λ_{max} (ϵ) 250 (25 500), 284 (5300); IR (neat) ν_{max} 3407, 3239, 2958, 1743, 1727, 1667, 1538, 1469, 1242, 1196, 1177, 694 cm^{-1}; EIMS *m/z* (rel intensity) 658/660/662 (2.1/1.4/0.3), 483 (7.6) 432/434/436 (9.5/6.4/1.8), 300/302/304 (8.0/5.5/1.2), 227 (100) 91 (87); high-resolution EIMS *m/z* 658.2207 (calcd for $C_{34}H_{40}Cl_2N_2O_7$, 0.6 mmu error); ^1H-NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.80 (2, d; 14.7), 6.66 (3, ddd; 14.7, 8.5 and 5.5), 2.38 (4, m), 2.53 (4, m), 4.97 (5, br dd; 10.4 and 6.2), 2.57 (6, m), 1.14 (6-Me, d; 6.7), 6.01 (7, dd; 15.9 and 8.7), 6.42 (8, d; 15.9), 7.28−7.34 (10/11/13/14, m), 7.22 (12, m); B 4.82 (2, m), 5.73 (2-NH, br d; 8.7), 3.02 (3, dd; −14.3 and 6.2), 3.10 (3, dd; -14.3 and 5.2), 7.14 (5/9, s), 5.79 (7-OH, s); C 2.73 (2, m), 1.21 (2-Me, d; 7.0), 3.17 (3, m), 3.60 (3, m), 6.81 (3-NH, br t; 6.7); D 4.84 (2, dd;10.0 and 3.2), 1.38 (3, ddd; −14.9, 10.2 and 3.2), 1.65 (3, m), 1.65 (4, m). 0.78 (4-Me, d; 6.5); 0.73 (5, d; 6.5). ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.5 (1), 125.4 (2), 141.2 (3), 36.4 (4), 77.6 (5), 42.3 (6), 17.3 (6-Me), 130.0 (7), 131.9 (8), 136.7 (9), 126.2 (10/14), 128.6 (11/13), 127.6 (12); B 171.0 (1), 53.2 (2), 35.0 (3), 130.4 (4), 129.1 (5/9), 121.0 (6/8), 146.7 (7); C 175.2 (1), 38.5 (2), 13.9 (2-Me), 41.6 (3), *D* 170.7 (1), 71.5 (2), 39.5 (3), 24.6 (4), 22.7 (4-Me), 21.2 (5).

Cryptophycin-21 (18): $[\alpha]_D$ +40.2° ($CHCl_3$, *c* 0.72); UV λ_{max} (ϵ) 204 (44 190), 218 (32 320), 244 (5190), 284 (1632); IR (neat) ν_{max} 3403, 3279, 2957, 1731, 1673, 1503, 1464, 1409, 1372, 1258, 1174, 1065, 1023, 889 cm^{-1}; EIMS *m/z* (relative intensity) 640/642 (10/4), 612 (5), 478 (15), 398 (40), 266 (33), 227 (76), 195 (95), 155 (100), 127 (90); high-resolution EIMS *m/z* 640.2550 (calcd for $C_{34}H_{41}ClN_2O_8$, 0.1 mmu error); ^1H NMR data: see Table 4; ^{13}C NMR data, see Table 5.

Cryptophycin-29 (19): $[\alpha]_D$ +22.2° ($CHCl_3$, *c* 1.13); UV λ_{max} (ϵ) 204 (45 600), 230 (17 200), 248 (16 500), 280 (2600); IR (neat) ν_{max} 3415, 3272, 2960, 1744, 1734, 1674, 1504, 1259, 1197, 1174, 1067, 694 cm^{-1}; EIMS *m/z* (rel intensity) 624/626 (2.6/1.1), 398/400 (44/15), 227 (100), 195/197 (50/16), 155/157 (59/20), 131 (63), 91 (95); high-resolution EIMS *m/z* 624.2607 (calcd for $C_{34}H_{41}ClN_2O_7$, −0.5 mmu error). ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.75 (2, dd; 15.3 and 1.1), 6.69 (3, ddd; 15.3, 10.1 and 5.3), 2.36 (4, m), 2.54 (4, m), 5.03 (5, ddd; 11.0, 6.4 and 1.8), 2.56 (6, m), 1.14 (6-Me, d; 6.8), 6.01 (7, dd; 15.8 and 8.8), 6.41 (8, d; 15.8), 7.28−7.33 (10/11/13/14, m), 7.22 (12, m); B 4.76 (2, m), 5.67 (2-NH, d; 8.6), 3.00 (3, dd; −14.4 and 7.4), 3.14 (3, dd; −14.4 and 5.9), 7.22 (5, d; 2.2), 3.87 (7-OMe, s), 6.83 (8, d; 8.4), 7.08 (9, dd; 8.4 and 2.2); C 2.55 (2-H_2, m), 3.44 (3, m), 3.55 (3, m), 6.89 (3-NH, br t; 5.7); D 4.90 (2, dd; 9.9 and 3.5), 1.34 (3, ddd; −15.4, 10.3 and 3.5), 1.63 (3, m), 1.63 (4, m). 0.76 (4-Me, d; 6.4), 0.72 (5, d; 6.4); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.6 (1), 125.2 (2), 141.5 (3), 36.4 (4), 77.1 (5), 42.3 (6), 17.3 (6-Me), 130.1 (7), 131.8 (8), 136.7 (9), 126.2 (10/14), 128.6 (11/13), 127.6 (12); B 170.9 (1), 53.8 (2), 34.9 (3), 129.9 (4), 131.0 (5), 122.4 (6), 153.9 (7), 56.1 (7-OMe), 112.2 (8), 128.4 (9); C 172.6 (1), 32.4 (2), 34.5 (3); D 170.5 (1), 71.5 (2), 39.7 (3), 24.4 (4), 21.2 (4-Me), 22.6 (5).

Cryptophycin-24 (20): $[\alpha]_D$ +48.8° ($CHCl_3$, *c* 0.63); UV ($CHCl_3$) λ_{max} (ϵ) 228 (19 006), 242 (8249), 274 (2351); IR (neat) ν_{max} 3400, 3284, 2959, 1732, 1678, 1652, 1514, 1248, 1178 cm^{-1}; EIMS *m/z* (rel intensity, assignment) 606 (2, M^+), 364 (7), 161 (55, $CH_3OC_6H_4$-$CH{=}CH{=}CO^+$), 121 (100, $CH_3OC_6H_4CH_2^+$), 91 (68); high-resolution EIMS *m/z* 606.2954 (calcd for $C_{34}H_{42}N_2O_8$, −1.3 mmu error); ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.69 (2, dd; 15.2 and 1.3), 6.70 (3, ddd; 15.2, 10.6 and 4.7), 2.43 (4, ddd; −14.3, 11.1 and 10.6), 2.53−2.59 (4, m), 5.20 (5, ddd; 11.1, 5.1 and 2.0), 1.76−1.82 (6, m), 1.14 (6-Me, d; 7.0), 2.92 (7, dd; 7.5 and 2.0), 3.68 (8, d; 2.0), 7.23 - 7.38 (10/11/12/13/14, m); B 4.73 (2, m), 5.58 (2-NH, d; 8.3), 3.03 (3, dd; −14.5 and 7.5), 3.14 (3, dd; −14.5 and 5.7), 7.11 (5/9, d; 8.6), 6.81 (6/8, d; 8.6), 3.78 (7-OMe, s); C 2.52−2.56 (2-H_2, m), 3.39−3.45 (3, m), 3.49−3.56 (3, m), 6.97 (3-NH, br t; 5.7); D 4.89 (2, dd; 9.9 and 3.5), 1.26−1.32 (3, m), 1.62 - 1.70 (3/4, m), 0.84 (4-Me, d; 6.1), 0.83 (5, d; 5.9); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.4 (1), 125.3 (2), 141.0 (3), 36.7 (4), 75.9 (5), 40.6 (6), 13.4 (6-Me), 63.0 (7), 59.0 (8), 136.7 (9), 125.6 (10/14), 128.7 (11/13), 128.5 (12); B 170.7 or 170.6 (1), 54.1 (2), 35.2 (3), 128.5 (4), 130.2 (5/9), 114.1 (6/8), 158.6 (7), 55.2 (7-OMe); C 172.8 (1), 32.4 (2), 34.2 (3); D 170.6 or 170.7 (1), 71.2 (2), 39.5 (3), 24.4 (4), 22.8 (4-Me), 21.3 (5).

Cryptophycin-18 (21): $[\alpha]_D$ +54.9° (MeOH, *c* 0.93); UV λ_{max} (ϵ) 208 (59 000), 228 (30 100), 248 (27 000), 280 (4150); IR (neat) ν_{max} 3411, 3271, 2966, 1746, 1728, 1668, 1505, 1463, 1258, 1178 cm^{-1}; EIMS *m/z* (rel intensity) 638/640 (4.5/1.1), 412/414 (59/19), 280(17), 227 (100); high-resolution EIMS *m/z* 638.2729 (calcd for $C_{35}H_{43}ClN_2O_7$, 2.9 mmu error); ^1H NMR, see Table 4; ^{13}C NMR, see Table 5.

Cryptophycin-50 (22): $[\alpha]_D$ +32.0° ($CHCl_3$, *c* 0.44); UV ($CHCl_3$) λ_{max} (ϵ) 242 (4933), 262 (3996, 274 (3719), 286 (2430), 332 (359); IR (neat) ν_{max} 3412, 3274, 2958, 1752, 1724, 1676, 1648, 1503, 1465, 1258, 1177; 1066, 753 cm^{-1}; EIMS *m/z* (rel intensity) 640/642 (4/2), 398/400 (11/4), 280/282 (10/3), 227 (17), 195/197 (57/18), 157 (20), 141 (31), 91 (100); high-resolution EIMS *m/z* 640.2531 (calcd. for $C_{34}H_{41}ClN_2O_8$, 2.0 mmu error); ^1H NMR data, see Table 4; ^{13}C NMR data, see Table 5.

Cryptophycin-49 (23): $[\alpha]_D$ +68.1° (MeOH, *c* 0.075); UV ($CHCl_3$) λ_{max} (ϵ) 246 (25 500), 284 (5200); IR (neat) ν_{max} 3401, 3282, 2962, 1744, 1728, 1668, 1540, 1505, 1464, 1258, 1198, 1177, 1066, 694 cm^{-1}; EIMS m/z (rel intensity) 624/626 (0.8/0.3), 398/400 (43/14), 227(78), 195/197 (58/26) 91 (100); high-resolution EIMS *m/z* 624.2650 (calcd for $C_{34}H_{41}ClN_2O_7$, −4.8 mmu error); ^1H NMR ($CDCl_3$) amino or

hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.77 (2, d; 14.1), 6.67 (3, m), 2.38 (4, m), 2.50 (4, m), 5.01 (5, m), 2.56 (6, m), 1.13 (6-Me, d; 6.5), 6.03 (7, dd; 15.8 and 8.6), 6.42 (8, d; 15.8), 7.29−7.35 (10/11/13/14, m), 7.23 (12; m); B 4.82 (2, m), 5.64 (2-NH, m), 3.06 (3, m), 3.13 (3, m), 7.22 (5, m), 3.87 (7-OMe, s), 6.83 (8, m), 7.08 (9, m); C 2.72 (2, m), 1.22 (2-Me, d; 6.7), 3.26 (3, m), 3.53 (3, m), 6.90 (3-NH, m); D 4.81 (2, dd; 8.8 and 3.9), 1.63 (3, m), 1.68 (3, m), 1.33 (4-H_2, m). 0.74 (5, t; 7.3).

Cryptophycin-19 (24): $[\alpha]_D$ +62.6° (MeOH, *c* 0.67); UV λ_{max} (ε) 204 (44 900), 230 (17 000), 248 (15 600), 280 (2500); IR (neat) ν_{max} 3413, 3272, 2966, 1745, 1726, 1672, 1504, 1258, 1199, 1178, 1066, 692 cm^{-1}; EIMS *m/z* (rel intensity) 624/626 (3.0/1.4), 398/400 (58/21), 280/282 (15/5), 227 (100), 195/197 (57/22); high-resolution EIMS *m/z* 624.2585 (calcd for $C_{34}H_{41}ClN_2O_7$, 1.7 mmu error); ^1H NMR data, see Table 4; ^{13}C NMR data, see Table 5.

Cryptophycin-54 (25): $[\alpha]_D$ +20.7° (MeOH, *c* 0.73); UV λ_{max} (ε) 204 (37 730), 218 (24 660), 234 (11 950), 284 (1980); EIMS *m/z* (relative intensity) 654/656 (17/10), 493 (5), 411/413 (12/4), 280 (16), 227 (25), 195/197 (45/25), 141 (30), 91 (100); high-resolution EIMS *m/z* 654.2686 (calcd for $C_{35}H_{43}ClN_2O_8$, 2.2 mmu error); ^1H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.73 (2, d; 15.4), 6.66 (3, ddd; 15.4, 9.7, 5.7), 2.46 (4, m), 2.53 (4, m), 5.16 (5, ddd; 11.0, 4.2, 1.7), 1.75−1.83 (6, m), 1.15 (6-Me, d; 6.8), 2.89 (7, dd; 7.4, 1.9), 3.70 (8, d; 1.9), 7.25 (10/14, m), 7.30−7.40 (11/12/13, m); B 4.82 (2, m), 5.63 (2-NH, d; 8.6), 3.03 (3, dd; −14.5, 7.3), 3.13 (3, dd; −14.5, 5.5), 7.21 (5, d; 2.2), 3.87 (7-OMe, s), 6.83 (8, d; 8.4), 7.07 (9, dd; 8.4, 2.2); C 2.73 (2, m), 1.22 (2-Me, d; 7.3), 3.26 (3, dt; −13.4, 6.8), 3.51 (3, ddd; −13.4, 6.8, 5.3), 6.88 (3-NH, br t; 6.8); D 4.73 (2, d; 4.2), 1.75−1.83 (3, m), 0.92 (3-Me, d; 6.8), 1.36−1.41 (4, m), 1.18-1.23 (4, m), 0.80 (5, t; 7.5). ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.3 (1), 125.2 (2), 141.0 (3), 36.6 (4), 76.3 (5), 40.6 (6), 13.2 (6-Me), 63.1 (7), 58.7 (8), 136.7 (9), 125.4 (10/14), 128.7 (11/13), 128.5 (12); B 170.9 (1), 53.5 (2), 35.0 (3), 129.8 (4), 131.0 (5), 125.4 (6), 153.9 (7), 56.1 (7-OMe), 112.2 (8), 128.4 (9); C 175.4 (1), 38.5 (2), 14.0 (2-Me), 41.3 (3); D 169.4 (1), 76.5 (2), 36.1 (3), 15.6 (3-Me), 24.0 (4), 11.2 (5).

Crystallographic Studies of 3. Colorless orthorhombic crystals of cryptophycin-3 (**3**) suitable for X-ray diffraction were obtained from a 20:5:1 mixture of $CHCl_3$/MeOH/H_2O. A crystal, 0.3 × 0.4 × 0.1 mm in dimension, was mounted on glass fibers with epoxy glue and centered on a Nicolet P3 automated diffractometer. The unit cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections. Reflections with 2θ in the range $4 \le 2\theta \le 40$ ($+h$, $+k$, $\pm l$) were measured by using the ω technique. A Ψ-scan absorption correction was applied. Crystal and instrument stabilities were monitored with a set of three standard reflections measured every 97 reflections; in all cases no significant variations were found. Ellipsoidal absorption corrections were applied for the data sets. Crystal data and relevant information for **3** are summarized as follows: space group *P*2(1)2(1)2(1) with $a = 7.588(5)$ Å, $b = 12.370(8)$ Å, $c = 38.16(2)$ Å; $v = 3582(4)$ Å^3; $Z = 4$; $\lambda = 0.710\,73$ Å (Mo Kα radiation); $\rho_{calc} = 1.185$ g/cm^3; $T = 298$ K; scan type = ω; scan rate = 1.5−15°/min; 2θ range = 4−40°; $\mu = 0.153$ mm^{-1}; minimum/maximum transmission coefficient = 0.754/0.744; reflections collected = 2025; number of independent reflections = 1964; number of unique data with $I > 3\sigma(I)$ = 1847; $R = \sum|F_o| - |F_c|/\sum F_o = 7.79\%$; $R_w = [w\sum(|F_o| - |F_c|)^2/\sum wF_o^2)]^{1/2} = 8.23\%$; goodness of fit (GOF) = $[w\sum(|F_o| - |F_c|)^2/N_o - N_v)]^{1/2} = 0.67$.

The structure was solved by direct methods using SHELTX version 4.2/360 (Siemens Instrument Corp.) and refined by full-matrix least-squares procedures. Due to the limitation of the allowed parameters (360 parameters), only selected non-hydrogen atoms were refined with anisotropic temperature coefficients. The hydrogen atoms were included by use of a riding model with C−H distances of 0.96 Å and isotropic thermal parameters fixed at 0.08 Å^2. In the final least-squares cycle, 356 parameters were refined.

Acid Hydrolysis of the Cryptophycins. (A) Marfey Analysis. The previously described procedure[2] was used except that the reversed-phase HPLC was carried out on a 25 cm × 4.6 mm C18 column (ALTEX, Ultrasphere 5 μm) using a flow rate of 1 mL/min for the eluant. The retention times of the FDAA standards were 39.8 min for L-(*S*)-3-(3,5-dichloro-4-hydroxyphenyl)alanine, 43.0 min for D-(*R*)-3-(3,5-dichloro-4-hydroxyphenyl)alanine, 44.0 min for L-(*S*)-3-(3-chloro-4-methoxyphenyl)alanine, 48.4 min for D-(*R*)-3-(3-chloro-4-methoxyphenyl)alanine, 37.0 min for L-(*S*)-3-(3-chloro-4-hydroxyphenyl)alanine (49.2 min for di-FDAA derivative), 41.0 min for D-(*S*)-3-(3-chloro-4-hydroxyphenyl)alanine (53.0 min for di-FDAA derivative), 42.8 min for L-(*S*)-*O*-methyltyrosine, 46.0 min for D-(*R*)-*O*-methyltyrosine, and 31.6 min for both (*R*)- and (*S*)-3-amino-2-methylpropionic acid. Each of the FDAA-derivatized acid hydrolysates of **10**, **18**, **22**, and **24** showed a HPLC peak at 48.4 min, indicating the presence of a D-(*R*)-3-(3-chloro-4-methoxyphenyl)alanine (D-CMPHE) unit in each of these chlorine-containing compounds. The FDAA-derivatized acid hydrolysate of **20** showed a HPLC peak at 46.0 min, indicating the presence of a D-(*R*)-*O*-methyltyrosine in this nonchlorinated cryptophycin, and the FDAA-derivatized acid hydrolysate of **13** showed an HPLC peak at 43.0 min, indicating the presence of a D-(*R*)-3-(3,5-dichloro-4-hydroxyphenyl)alanine unit in the dichlorinated cryptophycin. Coinjection of the derivatized unknown samples and authentic amino acids provided further proof.

(B) Chiral GC−MS Analysis. The previously described procedure[2] was used; however, the retention times were found to be slightly longer, viz. 26.87 and 27.47 min for the authentic samples of D-(*R*)- and L-(*S*)-*N*-(pentafluoropropionyl)-AMPA isopropyl esters, respectively. The retention time for the derivatized AMPA in the hydrolysates of **10**, **13**, **22**, and **24** was 26.87 min, indicating the presence of D-AMPA for unit C in each of these cryptophycins. The *N*-(pentafluoropropionyl)-β-alanine isopropyl ester and derivatized acid hydrolysates of **18** and **20** exhibited a peak in their GC chromatograms with a retention time of 28.0 min.

(C) Chiral TLC Analysis. Each of the acid hydrolysates of **10**, **13**, **18**, **20**, **22**, and **24** described above in parts A and B and the base hydrolysate of **21** were 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 acid hydrolysates of **10**, **13**, **18**, and **20** showed intense blue spots having R_f values of 0.66. Authentic L-leucic acid and D-leucic acid showed R_f values of 0.66 and 0.61, respectively. The isoleucic acid, in the acid hydrolysate of **21** showed a blue spot having a R_f value of 0.61. Authentic samples of L-isoleucic acid, D-isoleucic acid, L-*allo*-isoleucic acid, and D-*allo*-isoleucic acid showed R_f values of 0.61, 0.53, 0.64, and 0.56, respectively. The 2-hydroxy-3-methylbutyric acid from **24** and the L-2-hydroxy-3-methylbutyric acid standard showed blue spots with the same R_f value of 0.62, whereas the D-2-hydroxy-3-methylbutyric acid standard exhibited a R_f value of 0.56. The 2-hydroxyvaleric acid from **22** and the L-2-hydroxyvaleric acid standard had identical R_f values of 0.59, whereas the D-2-hydroxyvaleric acid standard had an R_f value of 0.49.

(*R*)- and (*S*)-3-Amino-2-methylpropionic acid (AMPA). Both compounds were prepared using procedures described in the literature.[2,9b,41]

D-(*R*)- and L-(*S*)-*O*-Methyltyrosine The standard samples were prepared from D- and L-tyrosine, respectively, by methylation of their amino acid moiety protected copper complex with dimethyl sulfate followed by deprotection with H_2S.[2]

D-(*R*)- and L-(*S*)-3-(3-Chloro-4-methoxyphenyl)alanine. Commercially available L-3-(3-chloro-4-hydroxyphenyl)alanine was converted to the corresponding *O*-methyl derivative as previously described.[2] The D isomer was prepared by direct dimethylation of D-3-chlorotyrosine with dimethyl sulfate to obtain D-(*R*)-3-(3-chloro-4-methoxyphenyl)alanine methyl ester which was then hydrolyzed to the free amino acid.[5]

(*R*)- and L-(*S*)-3-(3,5-Dichloro-4-hydroxyphenyl)alanine. A commercial sample of D-tyrosine (100 mg) in glacial acetic acid (0.5 mL) was treated with excess sulfuryl chloride (2 mL) at room temperature.

(41) Griffith, O. W.; Campbell, E. B.; Pirkle, W. H.; Tsipouras, A.; Hyun, M. H. *J. Chromatogr.* **1986**, *362*, 345−52.

(42) Signals for OH- and NH-bearing carbons generally show isotope shifts of 0.1−0.2 ppm compared with the corresponding OD- and ND-bearing carbon signals. Isotope shifts have been used to distinguish OH- and NH-bearing carbon signals from other types of O- and N-bearing carbon signals in the structure determination of natural products such as gambieric acids (Nagai, H.; Torigoe, K.; Satake, M.; Murata, M.; Yasumoto, T.; Hirota, H. *J. Am. Chem. Soc.* **1992**, *114*, 1102−3) and zooxanthellatoxin-A (Nakamura, H.; Asari, T.; Murai, A.; Kondo, T.; Yoshida, K.; Ohizumi, Y. *J. Org. Chem.* **1993**, *58*, 313−4).

After 5 h, the reaction mixture was evaporated under reduced pressure, the residue redissolved in 2 mL of water, and the pH of the resulting solution adjusted to 4 with 1 N NaOH. A white precipitate appeared which crystallized from water to give D-(*R*)-3-(3,5-dichloro-4-hydroxyphenyl)alanine in 80% yield. L-(*S*)-3-(3,5-dichloro-4-hydroxyphenyl)-alanine was prepared from L-(*S*)-tyrosine using the same procedure.

D- and L-Isoleucic acid and D- and L-*allo*-Isoleucic acid. D- and L-isoleucic acids and D-*allo*-isoleucic acid were available commercially as their sodium salts. L-*allo*-Isoleucic acid was synthesized by treating L-*allo*-isoleucine (100 mg) in 4 N HCl (1 mL) with excess sodium nitrite (0.5 gm in 1 mL water) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 4 h and at room temperature for overnight. The solution was extracted repeatedly with ethyl ether and the ether extract evaporated to yield L-*allo*-isoleucic acid which was converted into its sodium salt and purified by reversed phase HPLC (Econosil C18, 250 × 4.5 mm, 7:3 MeOH/H_2O).

2-Hydroxyvaleric Acid and 2-Hydroxy-3-methylbutyric Acid. The racemic acids and L-2-hydroxy-3-methylbutyric acid were obtained commercially.

Hydrolysis of 1. A solution of cryptophycin-1 (**1**, 60 mg) in 1 mL of constant boiling HCl was heated at 90 °C for 12 h and then evaporated in vacuo. The hydrolysate was dissolved in water, and the solution was adjusted to pH 4−5 with sodium acetate. The solution was evaporated to dryness and the solid hydrolysate extracted repeatedly with CH_2Cl_2. The extract was evaporated and the residue subjected to reversed phase HPLC (Econosil C18, 10 μm, 25 cm × 10 mm, 35% H_2O/MeCN) to give L-leucic acid, $[\alpha]_D$ −21.1° (1 N NaOH, *c* 0.26) [lit.[10] $[\alpha]_D$ −26.9° (1 N NaOH, *c* 1.55)], and (4*S*)-3-benzyl-2,4-dimethyl-2-cyclopentenone (**32**). The insoluble portion of the hydrolysate was subjected to flash column chromatography on ODS silica to give the AMPA fraction on elution with 0.5% CF_3COOH/H_2O and the CMPHE fraction on elution with 20% H_2O/MeOH. The CMPHE fraction was evaporated and the residue purified successively by reversed phase HPLC (25% H_2O/MeOH) and cation exchange chromatography to give D-3-(3-chloro-4-methoxyphenyl)alanine, $[\alpha]_D$ +3.0° (1 N HCl, *c* 1.8) ($[\alpha]_D$ +3.6° (1 N HCl, *c* 1.6) for a synthetic sample). The AMPA fraction was evaporated, the residue redissolved in 10 mL of water, and the solution adjusted to pH 2 with dilute HCl. This solution was placed on a small cation exchange column (5 cm × 1 cm, Dowex-50 resin) and the column washed thoroughly with water to remove neutral compounds and salts. Elution with 5 mL of 4 N NH_4OH and evaporation of the solvent in the effluent gave D-3-amino-2-methylpropionic acid, $[\alpha]_D$ −16.5° (H_2O, *c* 1.8) [lit.[9] $[\alpha]_D$ −15.4° (H_2O, c 1)].

(4*S*)-3-Benzyl-2,4-dimethyl-2-cyclopentenone (**32**): $[\alpha]_D$ −5.3° ($CHCl_3$, *c* 1.8); EIMS *m/z* (relative intensity) 200 (62), 109 (73), 91 (100); high-resolution EIMS *m/z* 200.1223 (calcd for $C_{14}H_{16}O$, −2.1 mmu error); ^1H NMR ($CDCl_3$) δ (carbon position, multiplicity; *J* in Hz) 1.80 (2-Me, br s), 2.71 (4, m), 1.14 (4-Me, d; 7.0), 1.98 (5, dd; −18.8 and 2.0), 2.58 (5, dd; −18.8 and 6.8), 3.58 (6, d; −14.8), 3.92 (6, d; −14.8), 7.14 (8/12, m), 7.31 (9/11, m), 7.24 (10, m); ^{13}C NMR ($CDCl_3$) δ (carbon position) δ 209.2 (1), 136.6 (2), 8.4 (2-Me), 174.8 (3), 34.7 (4), 19.1 (4-Me), 43.0 (5), 34.7 (6), 137.4 (7), 128.8 (8/12), 128.6 (9/11), 126.8 (10).

Conversion of 8 to 3. To a solution of cryptophycin-30 (**8**, 0.9 mg) in 120 μL of dry pyridine at 0 °C was added 0.6 mg of TsCl. The mixture was stirred at rt for 96 h and then passed through a small silica column with EtOAc. The solvent was evaporated, and the residue was dissolved in dry toluene and treated with 3 μL of DBU. After the mixture was stirred for 3 h, the solvent was removed and the residue subjected to normal phase HPLC (Econosil silica, 250 × 4.6 mm, 5 μm, 3:2 EtOAc/hexane) to give cryptophycin C (**3**), identical in all respects with the natural product.

(*R*)-(+)- and (*S*)-(-)- Mosher Esters of 8. A solution of 1.5 mg of cryptophycin-30 (**8**), 1.0 mg of DMAP, and 5 μL of triethylamine in 300 μL of CH_2Cl_2 (distilled from P_2O_5) was treated with (*S*)-(+)-MTPA chloride (3.0 μL), and the mixture was stirred at rt for 12 h. 3-(Dimethylamino)propylamine (3 μL) was added, and stirring was continued for another 10 min. The reaction mixture was passed through a small silica column (1 g) with EtOAc, and the effluent was evaporated to dryness. The residue on further purification by normal phase HPLC with 3:7 ethyl acetate/hexane gave the (*R*)-(+)-Mosher ester of **8** (0.9 mg). The (*S*)-(−)-Mosher ester of **8** was prepared similarly using (*R*)-(−)-MTPA chloride. Samples of the *R* and *S* esters having the same concentration were examined by ^1H NMR spectroscopy at 500 MHz. The chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) for selected protons of the (*S*)-(-) and (*R*)-(+)-MTPA esters of **8** are shown in Figure 3a.

Saponification of 8 and 9 to 33. To a solution of 4 mg of cryptophycin-30 (**8**) in 1 mL of 2:3 water/acetone was added 20 μL of 1 N sodium hydroxide. After being stirred at rt for 5 h, the reaction mixture was neutralized to pH 7 with 1 N HCl and evaporated. The residue was subjected to reversed-phase HPLC (Econosil C18 10μ, 250 × 10mm, 1:3 water/methanol) to give **33** (2.9 mg). Using the same procedure, 1 mg of cryptophycin-26 (**9**) was treated with 6 μL of 1 N sodium hydroxide, and the product was separated to give 0.6 mg of **33**: FABMS *m/z* 561 $[MH]^+$, 583 $[MNa]^+$, 599 $[MK]^+$, 669 [M + thioglycerol]$^+$; ^1H NMR (MeOH-d_4) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 1.30 (2, m), 1.56 (2, m), 4.14 (3, m), 2.33 (4-H_2, m), 3.73 (5, dt; 8.9 and 4.2), 2.36 (6, m), 1.04 (6-Me, d; 6.8), 6.20 (7, dd; 15.9 and 8.5), 6.39 (8, d; 15.9), 7.35 (10/14, m), 7.25 (11/13, br t; 7.4), 7.15 (12, tt; 7.3 and 1.5); B 4.52 (2, dd; 9.1 and 5.7), 2.75 (3, dd; −14.1 and 9.1) 3.04 (3, dd; −14.1 and 5.7), 7.23 (5, d; 2.1), 3.82 (7-OMe, s), 6.94 (8, d; 8.5), 7.10 (9, dd; 8.5 and 2.1); C 2.52 (2, m), 1.11 (2-Me, d; 6.8), 3.20 (3, dd; −13.6 and 6.8), 3.34 (3, dd; −13.6 and 6.6); ^{13}C NMR (MeOD-d_4) unit δ (carbon position) A 172.6 (1), 40.6. (2), 68.4 (3), 42.7 (4), 74.1 (5), 43.8 (6), 14.8 (6-Me), 131.1 (7), 131.0 (8), 138.4 (9), 126.3 (10/14), 128.7 (11/13), 127.2 (12); B 173.2 (1), 55.4 (2), 36.8 (3), 130.9 (4), 132.0 (5), 122.5 (6), 154.6 (7), 55.8 (7-OMe), 112.7 (8), 128.9 (9); C 41.1 (2), 16.3 (2-Me), 43.6 (3).

Acetonide 34. To a stirred solution of **33** (2.9 mg) in 1.0 mL of 2,2-dimethoxypropane was added 3 μL of concd HCl at rt. After 12 h, finely powdered dry potassium carbonate (5 mg) was added to the solution and stirring was continued for another 3 h. The resulting mixture was filtered, the filtrate evaporated in vacuo, and residual crude product purified by reversed phase HPLC with 3:1 MeOH/H_2O to give **34** (2.2 mg); ^1H NMR (MeOH-d_4) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 2.25 (2, dd; −14.5 and 5.5), 2.38 (2, dd; −14.5 and 7.0), 4.21 (3, m), 1.16 (4, dt; −12.7 and 11.4), 1.35 (4, dt; −12.7 and 2.3), 3.83 (5, ddd; 11.4, 5.4 and 2.3), 2.30 (6, m), 1.07 (6-Me, d; 6.8), 6.19 (7, dd; 15.9 and 8.0), 6.36 (8, d; 15.9), 7.31 (10/14, br d, 7.8), 7.24 (11/13, br t; 7.8), 7.14 (12, br t; 7.8), 1.33 (eq acetonide-Me; s), 1.41 (ax acetonide-Me; s); B 4.56 (2, dd; 8.9 and 5.9), 2.77 (3, dd; −14.0 and 8.9), 3.01 (3, dd; −14.0 and 5.9), 7.22 (5, d; 2.1), 3.82 (7-OMe, s), 6.96 (8, d; 8.5), 7.10 (9, dd; 8.5 and 2.1); C 2.46 (2, m), 1.03 (2-Me, d; 7.2), 3.21 (3, dd; −13.3 and 6.6), 3.31 (3, dd; −13.3 and 7.0); ^{13}C NMR (CD_3OD) unit δ (carbon position) A 172.9 (1), 43.9 (2), 67.8 (3), 34.8 (4), 73.8 (5), 43.3 (6), 16.6 (6-Me), 133.2 (7), 131.5 (8), 139.1 (9), 127.1 (10/14), 129.5 (11/13), 128.0 (12), 100.2 (acetonide-C), 30.4 (eq acetonide-Me), 20.0 (ax acetonide-Me); B 173.1 (1), 55.8 (2), 38.1 (3), 131.7 (4), 131.8 (5), 123.2 (6), 155.4 (7), 56.6 (7-OMe), 113.5 (8), 129.7 (9); C 181.2(1), 42.2 (2), 15.9 (2-Me), 43.7 (3).

Conversion of 8 to 9. Cryptophycin-30 (**8**, 0.5 mg) in 125 μL of anhydrous pyridine was treated with 1 μL of thionyl chloride in a reaction vial at rt. After 3 days, the reaction mixture was evaporated under nitrogen and dried under vacuum. The residue was dissolved in CH_2Cl_2, the solution applied to a small silica column, and the product eluted with ethyl acetate. The EtOAc was evaporated and the residue purified by normal phase HPLC (Econosil silica, 250 × 4.6 mm, 5 μm, 1:1 ethyl acetate/hexane) to give cryptophycin-26 (**9**, 0.2 mg) and recovered **8** (0.2 mg).

Conversion of 9 to 8. A stirred solution of cryptophycin-26 (**9**, 0.5 mg) in 0.5 mL of DME was treated with 2 μL of 3:7 concentrated HBr/acetic acid. After 4 h, powdered dry potassium carbonate was added and stirring was continued for another 30 min. The reaction mixture was filtered, the filtrate evaporated, and the residue purified by HPLC (Econosil silica 10 μm, 250 × 10 mm, 1:1 EtOAc/hexane) to give cryptophycin-30 (**8**, 0.3 mg).

Saponification of 10 to 35. To a stirred solution of cryptophycin-28 (**10**, 1.3 mg) in 500 μL of 4:1 acetone/H_2O was added 5 μL of 1 N NaOH. After 12 h at rt, the solution was neutralized to pH 7 with 1 N HCl, and the solvent was evaporated. The residue was subjected to reversed phase HPLC on a Econosil C18 column with 3:1 MeOH/H_2O

to give **35** (0.9 mg): ^{1}H NMR (MeOH-d_4) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 6.00 (2, d; 15.5), 6.79 (3, dt; 15.5 and 7.3), 2.30 - 2.44 (4-H_2, m), 3.80 (5, m), 2.30 - 2.44 (6-H_2, m), 6.28 (7, dt; 15.8 and 7.3), 6.44 (8, d; 15.8), 7.35 (10/14, brd; 7.3), 7.26 (11/13; br t, 7.3), 7.16 (12, br t; 7.3); B 4.58 (2, dd, 8.6 and 6.2), 2.81 (3, dd; −13.7 and 8.6), 3.04 (3, dd; −13.7 and 6.2), 7.25 (5, d; 2.1), 3.82 (7-OMe, s), 6.95 (8, d; 8.6), 7.12 (9, dd; 8.6 and 2.1); C 2.48 (2, m), 1.02 (2-Me, d; 7.3), 3.20 (3, dd; −13.2 and 6.7), 3.34 (3, dd; −13.2 and 6.9).

(*R*)-(+)- and (*S*)-(-)- Mosher Esters of 35. A solution of **35** (0.4 mg), DMAP (0.4 mg), and triethylamine (2 μL) in 150 μL of CH_2Cl_2 (distilled from P_2O_5) was treated with (*S*)-(+)-MTPA chloride (1 μL), and the solution was stirred at rt for 12 h. 3-(Dimethylamino)-propylamine (1 μL) was added, and the solvent was evaporated. The residue was dissolved in 33:67 MeOH/H_2O and applied to a small C18 column (1 g). The column was washed with 5 mL of 33:67 MeOH/H_2O and the product eluted with 5 mL of CH_3OH. Evaporation of the solvent left a residue which was further purified by reversed phase HPLC with 85:15 MeOH/H_2O to give the (*R*)-(+)-Mosher ester of **35** (0.3 mg). The (*S*)-(−)-Mosher ester was similarly prepared by treating **35** with (*R*)-(−)-MTPA chloride. The NMR samples of the (*R*)- and (*S*)-MTPA esters were adjusted to the same concentration in CD_3OD, and the 500 MHz ^{1}H NMR spectra were obtained. Chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) for selected protons of the *S* and *R* esters are depicted in Figure 3b.

Conversion of 10 to 11 and 36. A mixture of 2 mg of cryptophycin-28 (**10**) and 1.2 equiv of *m*-CPBA in 0.5 mL of CH_2Cl_2 was stirred at room temperature for 1 h, at which time 250 μL of phosphate buffer (pH 8) was added. Stirring was continued for an additional hour, and then the aqueous phase was discarded. The organic layer was treated with another 300 μL of phosphate buffer, along with 75 μL of dimethyl sulfide. After another hour of stirring, the aqueous phase was discarded and the organic layer evaporated. The residue was dried in vacuo for 24 h and subjected to reversed-phase HPLC (C-18, 10 μm, 10 mm × 250 mm, 60% aqueous CH_3CN as mobile phase) to give 1.2 mg of a 2:1 mixture of **11** and the *S,S*-epoxide, cryptophycin-107 (**36**).

Cryptophycin-107 (**36**): ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 5.79 (2, d; 15.4), 6.70 (3, m), 2.55 (4, m), 2.41 (4, m), 5.32 (5, m), 1.96−2.08 (6-H_2, m), 2.96 (7, ddd; 8.7, 4.5, 1.9), 3.61 (8, d; 1.9), 7.30−7.36 (10/11/13/14, m), 7.20−7.23 (12, m); B 4.83 (2, m), 5.68 (2-NH, d; 8.6), 3.03 (3, dd; −14.3, 7.5), 3.16 (3, dd, −14.3, 5.3), 7.21 (5, d; 1.5), 3.87 (7-OMe, s), 6.84 (8, d; 8.3), 7.08 (9, dd; 8.3, 1.5); C 2.72 (2, m), 1.23 (2-Me, d; 7.3), 3.31 (3, m), 3.50 (3, m), 6.96 (3-NH, br t; 6.0); D 4.86 (2, m), 1.46 (3, m), 1.64−1.75 (3/4, m), 0.86 (4-Me, d; 6.5), 0.87 (5, d; 6.5).

Conversion of 11 to 37. Cryptophycin-40 (**11**, 1.3 mg) in 200 μL of DME was treated with 1 μL of concentrated HCl and stirred for 30 min. Solid K_2CO_3 was then added, the mixture was stirred an additional 1 h and filtered, and the filtrate was evaporated under N_2. Reversed phase HPLC purification of the residual solid (C18, 10 μm, 10 × 250 mm, 65% aqueous CH_3CN at 3 mL/min, t_R 16 min) gave 0.8 mg of pure cryptophycin-63 (**37**). The *R*-isomer was not obtained: EIMS *m/z* (relative intensity) 640/642 (9/3) [M-HCl]$^+$, 412/414 (6/3), 280/282 (16/14), 213 (35), 195/197 (65/19), 91 (100); high-resolution EIMS *m/z* 640.2502 (calcd for $C_{34}H_{41}ClN_2O_8$, 4.6 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 5.75 (2, d; 15.4), 6.68 (3, ddd; 15.4, 9.3, 5.1), 2.45 (4, m), 2.37 (4, m), 5.32 (5, br t; 10.0), 2.13 (6, m), 1.80 (6, m), 3.90 (7, m), 4.77 (8, d; 6.2), 7.32-7.41 (10/11/12/13/14, m); B 4.81 (2, m), 5.62 (2-NH, d; 8.0), 3.15 (3, dd; −14.5, 5.5), 3.02 (3, dd; −14.5, 7.1), 7.22 (5, d; 1.5), 3.87 (7-OMe, s), 6.84 (8, d; 8.2), 7.08 (9, dd; 8.2, 1.5); C 2.73 (2,m), 1.22 (2-Me, d; 7.1), 3.52 (3, ddd; −13.5, 8.6, 5.9), 3.27 (3, ddd; −13.5, 6.6, 5.9), 6.92 (3-NH, br t; 5.9), *D* 4.86 (2, dd;, 9.3, 3.8), 1.60−1.76 (3/4, m), 1.48 (3, m), 0.96 (4-Me, d; 6.7), 0.93 (5, d; 6.7).

(*R*)-(+)- and (*S*)-(−)- Mosher Esters of 37. Two samples of **37** (1.0 mg each) were treated with (*S*)-(+)-MTPA chloride and (*R*)-(+)-MTPA chloride as described above for **8** and **35**. Purification of the Mosher esters was accomplished by normal phase HPLC, and after each sample was adjusted to the same concentration in CD_3OD the NMR spectra were obtained. Chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) for selected protons of the *S* and *R* esters are depicted in Figure 3c.

Conversion of 12 to 1, 13 to 14, and 15 to 3. Cryptophycin-16 (**12**, 1 mg) in 0.5 mL of dry acetone was treated with 50 μL of methyl iodide and 20 mg of finely powdered dry potassium carbonate in a reaction vial at 70 °C for 4 h. The reaction mixture was filtered through an Alltech 13 mm, 0.2 μm nylon filter and the filtrate evaporated to dryness. The residue was placed on a small silica column (500 mg) and eluted with CH_2Cl_2 and EtOAc. The EtOAc fraction was evaporated to give **1** (0.8 mg), identical in all respects with the natural product. Using the same procedure, cryptophycin-23 (**13**, 0.7 mg) was methylated to cryptophycin-31 (**14**) (0.6 mg after normal phase HPLC with 43:57 EtOAc/hexane). Similarly, cryptophycin-17 (**15**, 1 mg) was converted to **3** (0.6 mg after reversed-phase HPLC with 3:1 MeCN/H_2O).

Conversion of 18 to 19 and 13 to 17. A solution of 16 mg of freshly generated bis(cyclopentadienyl)titanium(III) chloride[21] in dry THF was added to a solution of 12 mg of cryptophycin-21 (**18**) in 1 mL of dry THF and vigorously stirred under argon. After 12 h, the reaction was quenched with 100 μL of saturated monobasic sodium phosphate solution. The mixture was diluted after 5 min with 20 mL of water and extracted thoroughly with EtOAc (4 × 40 mL). The lipophilic extract was dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by reversed phase HPLC (Econosil C18, 10 μm, 250 × 10 mm, 11:39 water/MeCN) to give cryptophycin-29 (**19**, 5 mg), identical in all respects with the natural product. Using the same procedure, cryptophycin-23 (**13**, 1 mg) in 0.5 mL of THF was treated with 2 mg of the titanium reagent. After 12 h, 30 μL of saturated sodium phosphate was added, followed 5 min later by 10 mL of water. The reaction mixture was extracted with EtOAc (3 × 20 mL), and the combined organic layers were evaporated to dryness. The residue was subjected to normal phase HPLC (Econosil silica 5 μm, 250 × 4.6 mm, 2:3 EtOAc/hexane) to give cryptophycin-45 (**17**, 0.3 mg), identical in all respects with the natural product.

Saponification of 3 to 31 and 21 to 38. To a stirred solution of 5 mg of cryptophycin-3 (**3**) in 2:3 H_2O/DME was added 31 μL (4 equiv) of 1 N NaOH at room temperature. After 2 h, the pH of the reaction mixture was carefully adjusted to 7−8 with 1 N HCl, and the solution was evaporated to dryness under nitrogen. The residue was subjected to reversed phase HPLC (Econosil C18, 10 μm, 25 cm × 10 mm, 1:3 H_2O/MeOH, 3 mL/min) to obtain **31** (3.5 mg). Using the same procedure 2 mg of cryptophycin-18 (**21**) in 0.5 mL of 3:2 DME/H_2O was treated with 13 μL (4 equiv) of 1 N NaOH. After 8 h, the reaction mixture was subjected to same isolation procedure to give **31** (0.7 mg), **38** (0.5 mg), and isoleucic acid. In another experiment, 8 mg of **21** was treated with 75 μL of 1 N NaOH. After 20 h, the reaction mixture was processed using the same isolation procedure to give 5.1 mg of **31** and 1.0 mg of isoleucic acid.

Isoleucic acid: $[\alpha]_D$ −16.7° (1 N NaOH, *c* 1.2) (lit.[24] $[\alpha]_D$ −11.8° (H_2O, *c* 4.62); ^{1}H NMR (CD_3OD) δ (carbon position, multiplicity; J in Hz) 0.89 (5, t; 6.0), 0.98 (3-Me, d; 6.5), 1.23 (4, m), 1.46 (4, m), 1.82 (3, m), 3.89 (2, m). ^{13}C NMR ($CDCl_3$) δ 180.3, 77.9, 40.3, 24.8, 16.3, 12.3.

Compound **31**: FABMS *m/z* 543 [MH]$^+$, 565 [MNa]$^+$, 581 [MK]$^+$, 651 [M + thioglycerol]$^+$; ^{1}H NMR (MeOH-d_4) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 5.98 (2, d; 15.5), 6.79 (3, dt; 15.5 and 7.5), 2.30 (4, m), 2.37 (4, m), 3.64 (5, dt; 7.9 and 4.6), 2.39 (6, m), 1.02 (6-Me, d; 7.1), 6.22 (7, dd; 16.0 and 8.5), 6.40 (8, d; 16.0), 7.35 (10/14, br d; 7.6), 7.26 (11/13, br t; 7.6), 7.16 (12, br t; 7.6); B 4.58 (2, dd; 8.5 and 6.4), 2.81 (3, dd; −13.8 and 8.5) 3.04 (3, dd; −13.8 and 6.4), , 7.25 (5, d; 2.1), 3.82 (7-OMe, s), 6.95 (8, d; 8.4), 7.12 (9, dd; 8.4 and 2.1); C 2.50 (2, m), 1.13 (2-Me, d; 6.9), 3.18 (3, dd; −13.4 and 6.8), 3.37 (3, dd; −13.4 and 6.8); ^{13}C NMR (MeOH-d_4) unit δ (carbon position) A 168.2 (1), 126.1 (2), 143.5 (3), 38.8 (4), 75.3 (5), 44.2 (6), 15.6 (6-Me), 132.6 (7), 132.1 (8), 139.1 (9), 127.1 (10/14), 129.5 (11/13), 128.0 (12); B 173.4 (1), 56.2 (2), 38.0 (3), 131.7 (4), 131.9 (5), 123.2 (6), 155.4 (7), 56.6 (7-OMe), 113.5 (8), 129.8 (9); C 179.9 (1), 41.4 (2), 17.5 (2-Me), 43.4 (3).

Compound **38**: FABMS *m/z* 657 [MH]$^+$, 679 [MNa]$^+$, 695 [MK]$^+$, 765 [M + thioglycerol]$^+$, 789 [MCs]$^+$; ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 5.77 (2,

d; 14.9), 6.73 (3, m), 2.37−2.50 (4-H_2, m), 5.15 (5, m), 2.60 (6, m), 1.12 (6-Me, d; 7.1), 6.06 (7, dd; 15.9 and 8.5), 6.42 (8, d; 15.9), 7.21−7.34 (10/11/12/13/14); B 4.80 (2, m), 2.92 (3, dd; −13.9 and 6.5), 3.04 (3, dd; −13.9 and 7.1), 7.17 (5, d; 1.7), 3.84 (7-OMe, s), 6.81 (8, d; 8.5), 7.03 (9, dd; 8.5 and 1.7); C 2.70 (2, m), 1.17 (2-Me, d; 7.1), 3.14 (3, m), 3.51 (3, m); D 4.13 (2, d; 3.4), 1.79 (3, m), 0.96 (3-Me, d; 6.8), 1.22 (4, m), 1.31 (4, m), 0.78 (5, t; 7.3); ^{13}C NMR ($CDCl_3$) δ 177.9, 174.9, 170.9, 154.0, 141.0, 136.9, 131.9, 130.9, 129.7, 128.6 (2xC), 128.4, 127.6, 126.2 (2xC), 125.5, 122.4, 112.2, 74.9, 56.1, 54.1, 41.8, 41.1, 39.2, 38.6, 36.5, 35.2, 23.4, 16.8, 15.3, 14.8, 11.5.

Conversion of 21 to 25 and 39. Using the same procedure described above for the conversion of **10** to **11**, cryptophycin-18 (**21**, 4 mg) was converted to a 2:1 mixture of epoxides which could be separated in the HPLC step to give 1.3 mg of **25** and 0.6 mg of the *S,S*-epoxide, cryptophycin-101 (**39**).

Cryptophycin-101 (**39**): EIMS *m/z* (relative intensity) 654/656 (7/4), 533 (5), 412/414 (12/3), 280/282 (12/5), 195/197 (56/25), 155 (54), 91 (100); high-resolution EIMS *m/z* 654.2706 (calcd for $C_{35}H_{43}ClN_2O_8$, 0.2 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.81 (2, dd; 15.5 and 0.6), 6.68 (3, ddd; 15.5, 9.4 and 5.8), 2.54 (4, m), 2.69 (4, m), 5.18 (5, ddd; 12.9 and 4.3, 1.5), 1.75 (6, m), 1.07 (6-Me, d; 7.2), 2.92 (7, dd; 8.1 and 2.0), 3.60 (8, d; 2.0), 7.24 (10/14, m), 7.32−7.38 (11/12/13, m); B 4.84 (2, m), 5.70 (2-NH, d; 8.7), 3.05 (3, dd; −14.5 and 7.1), 3.16 (3, dd; −14.5 and 5.5), 7.24 (5, d; 2.3), 3.88 (7-OMe, s), 6.85 (8, d; 8.5), 7.09 (9, dd; 8.5 and 2.3); C 2.75 (2, m), 1.24 (2-Me, d; 7.2), 3.24 (3, dt, −13.6 and 5.7), 3.57 (3, ddd; −13.6, 5.7 and 4.0), 6.88 (3-NH, br t; 5.7); D 4.84 (2, d; 4.3), 1.98 (3, m), 0.97 (3-Me, d; 6.8), 1.44 (4, m), 1.25 (4, m), 0.87 (5, t; 7.5).

Conversion of 1 to 30 and 18 to 42. Compounds **1** and **18** (5 mg each) were treated with 8 mL of pH 11 Na_2CO_3 solution in 3:2 DME/H_2O. The pH was maintained by addition of Na_2CO_3 in DME/H_2O. At 10 min intervals, 5 μL aliquots were removed and analyzed by reversed phase HPLC (C18, 5 μm, 4.6 × 250 mm, 80% aqueous acetonitrile at 1.5 mL/min) to determine the rate of disappearance of starting material. The retention times (t_R) were found to be 1.2 min for **1**, 3.4 min for **30**, 1.4 min for **18**, and 3.2 min for **42**. When these data were plotted as the log of percentage of starting material remaining vs progress of reaction, a linear relationship was observed. From the plots, the half-lives of cryptophycin-1 (**1**) and cryptophycin-21 (**18**) were determined to be 50 and 15 min, respectively.

Cryptophycin-13 (**30**): EIMS *m/z* (relative intensity) 672/674 (1/0.3), 297/299 (25/9), 250/252 (12/4), 155 (100); high-resolution EIMS *m/z* 672.2824 (calcd for $C_{35}H_{45}ClN_2O_9$, −1.0 mmu error); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.80 (2, d; 15.8), 6.70 (3, ddd; 15.8, 9.0 and 5.4), 2.63 (4, dt; −14.9 and 8.8), 2.56 (4, m), 5.13 (5, m), 1.77−1.86 (6, m), 1.10 (6-Me, d; 6.9), 2.90 (7, dd; 7.1 and 2.0), 3.70 (8, d; 2.0), 7.23 (10/14, brd; 7.3), 7.28−7.36 (11/12/13, m); B 4.82 (2, dt; 6.6 and 7.4), 2.88 (3, dd; −14.0 and 7.4), 3.03 (3, dd; −14.0 and 6.6), 7.17 (5, d; 2.2), 3.84 (7-OMe, s), 6.80 (8, d; 8.4), 7.02 (9, dd; 8.4 and 2.2); C 2.67 (2, m), 1.14 (2-Me, d; 7.2), 3.08 (3, m), 3.52 (3, m); D 4.14 (2, dd; 9.9 and 3.8), 1.38 (3, m), 1.45 (3, m), 1.77−1.86 (4, m), 0.86 (4-Me, d; 6.7), 0.88 (5, d; 6.5).

Compound **42**: ^{1}H NMR (CD_3OD) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 6.02 (2, d; 15.3), 6.65 (3, ddd; 15.3, 9.2 and 6.8), 2.52 (4, m), 2.62 (4, m), 5.08 (5, m), 1.78 (6, m), 1.11 (6-Me, d; 6.8), 2.94 (7, dd; 7.7 and 1.9), 3.75 (8, d; 1.9), 7.25−7.37 (10/11/12/13/14, m); B 4.55 (2, m), 3.03 (3, dd; −14.0 and 7.2), 2.80 (3, dd; −14.0 and 6.7), 7.23 (5, br d; 2.1), 3.82 (7-OMe, s), 6.94 (8, d; 8.3), 7.10 (9, dd; 8.3 and 2.1); C 2.34 (2-H_2, m), 3.37 (3-H_2, m); D 4.06 (2, dd; 9.6 and 4.3), 1.41 (3, m), 1.34 (3, m), 1.75 (4, m), 0.85 (4-Me, d; 6.6), 0.84 (5, d; 6.6).

Conversion of 30 to 29. Cryptophycin-13 (**30**, 5 mg) was treated with 2 mL of NaOH solution in 3:2 DME/H_2O having a pH of 11.5. The pH was maintained at pH 11.5 by addition of 0.5 N NaOH in 3:2 DME/H_2O. The rate of disappearance of the starting material was monitored by analyzing 5 μL aliquots of the reaction mixture at 10 min intervals by reversed phase HPLC (Ultrasphere ODS, 5 μm, 25 cm × 4.6 mm, 25:75:0.1 $H_2O/CH_3OH/CF_3COOH$ at 1 mL/min). The retention times (t_R) were found to be 5.0 min for **29** and10.1 min for **30**. The semi-log linear plot exhibited a half life of 54 min for cryptophycin-13 (**30**).

Cryptophycin-12 (**29**): ^{1}H NMR (CD_3OD) see elsewhere;[2] ^{13}C NMR (CD_3OD) unit δ (carbon position) A 168.0 (1), 126.7 (2), 142.2 (3), 38.0 (4), 84.0 (5), 47.7 (6), 14.3 (6-Me), 85.9 (7), 85.9 (8), 142.8 (9), 127.1 (10/14), 129.3 (11/13), 128.6 (12); B 173.4 (1), 56.3 (2), 38.0 (3), 131.6 (4), 131.9 (5), 123.3 (6), 155.4 (7), 56.6 (7-OMe), 113.4 (8), 129.8 (9); C 179.0 (1), 40.8 (2), 15.3 (2-Me), 43.1 (3). ^{1}H NMR (CD_3OH): *A* 168.1 (1), 86.0 (7); B 173.5 (1), 56.4 (2); C 179.2 (1), 43.3 (3).[42]

Cryptophycin-12 *O*-Acetate. Cryptophycin-12 (**29**, 1 mg) was treated with acetic anhydride (300 μL) and pyridine (20 μL) at room temperature. After 5 h, the solvent was evaporated and the residue purified by reversed phase HPLC (Econosil C18, 25 cm × 10 mm, 10 μm, 30:70:1 $H_2O/CH_3OH/CF_3COOH$, 3 mL/min) to give cryptophycin-12 *O*-acetate (0.9 mg): ^{1}H NMR (CD_3OD) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 6.08 (2, br d; 15.5), 6.84 (3, dt; 15.5 and 7.4), 2.52−2.63 (4-H_2, m), 4.00 (5, dt; 5.1 and 7.1), 2.14 (6, m), 0.99 (6-Me, d; 6.8), 5.00 (7, dd; 5.6 and 5.0), 2.06 (7-OCOMe, s), 4.93 (8, d; 5.0), 7.38 (10/14, m), 7.33 (11/13, m), 7.26 (12, m); B 4.58 (2, dd; 8.3 and 6.6), 2.83 (3, dd; −13.8 and 8.3), 3.02 (3, dd; −13.8 and 6.8), 7.25 (5, d; 2.2), 3.82 (7-OMe, s), 6.95 (8, d; 8.4), 7.13 (9, dd; 8.4 and 2.2); C 2.52−2.63 (2, m), 1.02 (2-Me, s), 3.14 (3, dd; −13.6 and 7.2), 3.43 (3, dd; −13.6 and 6.5).

Conversion of 1 to 26. Method I. To a solution of **1** (500 mg) in 3 mL of DME was added 60 μL of concentrated HCl.[43] The mixture was stirred at room temperature for 18 h, solid K_2CO_3 (50 mg) was added, and the mixture was stirred for an additional 2 h and then filtered and evaporated. The residue was subjected to reversed phase HPLC on a 250 mm × 22 mm C18 column (Alltech Econosil, flow rate 5 mL/min, 20 mg/injection, UV detection at 254 nm) using 3:1 MeCN/H_2O to give 433 mg of cryptophycin-8 (**26**, t_R 25 min).

Method II. To a solution of **1** (45 mg) in 1 mL of chloroform cooled to −60 °C was added 17 μL of trimethylsilyl chloride. After 2 h, the mixture was warmed to room temperature and evaporated. Reversed phase HPLC of the residue as described above led to 42 mg of **26**.

Conversion of 1 to 41. A solution of **1** (102 mg) in 3 mL of DME was treated slowly at −75 °C with 35 μL of 30% of HBr in acetic acid and then allowed to slowly warm to room temperature. After 12 h, the excess acid was neutralized with solid K_2CO_3. The mixture was filtered and evaporated and the residue subjected to reversed-phase HPLC (Econosil C18, 25 cm × 22 mm, 10 μm, 30% H_2O/CH_3CN, 6 mL/min) to give cryptophycin-25 (**45**) (t_R 37.2 min, 83.2 mg).

Cryptophycin-25 (**45**): EIMS *m/z* (relative intensity) 654/656 (0.5/0.3, M^+ − HBr), 412/414 (1.4/0.4), 195/197 (11/3), 91 (100); ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; *J* in Hz) A 5.80 (2, d; 15.3), 6.70 (3, ddd; 15.3, 9.7 and 5.5), 2.38 (4, ddd; 14.0, 10.6 and 9.7), 2.68 (4, brdd; 14.0, 5.5), 5.11 (5, ddd; 10.6, 9.0 and 1.5), 2.58 (6, m), 1.03 (6-Me, d; 7.0), 4.19 (7, brd; 9.9), 4.73 (8, d; 9.9), 7.32−7.42 (10/11/12/13/14, m); B 4.81 (2, m), 5.68 (2-NH, d; 8.6), 3.03 (3, dd; 14.4 and 7.4), 3.16 (3, dd; 14.5 and 5.5), 7.23 (5, d; 2.2), 3.88 (7-OCH_3, s), 6.85 (8, d; 8.3), 7.09 (9, dd; 8.4 and 2.2); **C** 2.74 (2, m), 1.23 (2-Me, d; 7.2), 3.26 (3, dt; 13.6, 6.8), 3.53 (3, ddd; 13.6, 5.0 and 4.2), 6.93 (3-NH, brdd; 6.8 and 5.0); **D** 4.93 (2, dd; 10.1 and 3.1), 1.74 - 1.83 (3/4, m), 1.49 (3, m), 0.95 (4-Me/5, d; 6.6); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.6 (1), 125.2 (2), 141.6 (3), 36.3 (4), 76.4 (5), 39.1 (6), 8.6 (6-Me), 73.5 (7), 53.8 (8), 138.7 (9), 128.3 (10/14), 129.1 (11/12/13); B 171.1 (1), 53.7 (2), 35.0 (3), 130.0 (4), 131.0 (5), 122.3 (6), 153.9 (7), 56.1 (7-OCH_3), 112.2 (8), 128.4 (9); **C** 175.3 (1), 38.3 (2), 14.1 (2-Me), 41.2 (3); **D** 170.6(1), 71.3 (2), 39.7 (3), 24.7 (4), 21.5 (4-Me), 23.1 (5).

Conversion of 1 to 43 and 44. Cryptophycin-1 (**1**, 5 mg) in 1 mL of DME was treated with 20 μL of 30% methylamine in water at rt for 60 h. The reaction mixture was evaporated and the residue subjected to reversed phase HPLC (Econosil C18, 250 mm × 10 mm column, 10 μm, 65% MeCN in water, 2.5 mL/min flow rate) to give acyclic cryptophycin-65 (**43**) (1.9 mg), cryptophycin-66 (**44**) (1.2 mg), the *N*-methylamide of leucic acid (**45**), and 1.1 mg of starting material.

(43) There is an error in the experimental procedure described previously for the preparation of **26** from **1**.[2] Anhydrous DME was actually used as the solvent. In 2:1 DME/H_2O, comparable amounts of **26** and **40** are formed.

Cryptophycin-65 (**43**): ^{1}H NMR ($CDCl_3$) amino or hydroxy acid unit δ (carbon position, multiplicity; J in Hz) A 5.84 (2, d; 15.3), 6.69 (3, ddd; 15.3, 8.7 and 6.4), 2.59 (4, dddd; −15.1, 6.3, 4.0 and 1.5), 2.46 (4, dt; −15.1 and 8.4), 5.10 (5, ddd; 8.5, 6.0 and 3.8), 1.80-1.88 (6, m), 1.13 (6-Me, d; 6.7), 2.91 (7, dd; 7.4 and 2.0), 3.69 (8, d; 2.0), 7.23−7.26 (10/14, m), 7.30−7.37 (11/12/13, m); B 4.55 (2, dt; 7.6 and 7.1), 6.20 (2-NH, br d), 2.95 (3-H_2, d; 7.1), 7.17 (5, d; 2.1), 3.86 (7-OMe, s), 6.84 (8, d; 8.4), 7.03 (9, dd; 8.4 and 2.1); C 2.75 (1-NMe, d; 4.9), 6.65 (1-NH, br m), 2.52 (2, dqd; 9.0, 7.0 and 4.9), 1.05 (2-Me, d; 7.0), 3.37 (3, ddd; −13.5, 6.5 and 4.9), 3.12 (3, ddd; −13.5, 9.0 and 5.7), 6.05 (3-NH, br t); D 4.09 (2, dd; 9.7 and 4.0), 1.47 (3, ddd; −13.7, 9.7 and 5.0), 1.39 (3, ddd; −13.7, 9.2 and 4.0), 1.80−1.88 (4, m), 0.90 (5, d; 6.5), 0.88 (4-Me, d; 6.7); ^{13}C NMR ($CDCl_3$) unit δ (carbon position) A 165.1 (1), 126.5 (2), 139.4 (3), 34.8 (4), 75.2 (5), 39.6 (6), 13.0 (6-Me), 63.3 (7), 58.8 (8), 136.8 (9), 125.7 (10/14), 128.7 (11/13), 128.5 (12); B 171.2 (1), 54.7 (2), 37.1 (3), 129.4 (4), 131.0 (5), 122.4 (6), 154.1 (7), 56.1 (7-OMe), 112.2 (8), 128.4 (9); C 175.5 (1), 26.3 (1-NMe), 40.3 (2), 15.5 (2-Me), 42.8 (3); D 175.0 (1), 69.4 (2), 43.2 (3), 24.3 (4), 21.4 (5), 23.2 (4-Me).

Cryptophycin-66 (**44**): ^{1}H NMR (CD_3OD) amino or hydroxy acid unit δ (carbon position or group, multiplicity; J in Hz) A 6.01 (2, d; 15.5), 6.80 (3, dt; 15.5 and 7.5), 2.46 (4, m), 2.33 (4, dt; −14.5 and 7.5), 3.66 (5, br ddd; 7.5, 5.5 and 4), 1.51 (6, m), 1.11 (6-Me, d; 6.9), 2.91 (7, dd; 7.8 and 1.8), 3.74 (8, d; 1.8), 7.26−7.38 (Ph, m); B 4.51 (2, dd; 8.4 and 6.5), 3.01 (3, dd; −13.8 and 6.5), 2.81 (3, dd; −13.8 and 8.4), 7.25 (5, d; 1.7), 3.82 (7-OMe, s), 6.95 (8, d; 8.4), 7.12 (9, dd; 8.4 and 1.7); C 2.68 (1-NMe, s), 2.47 (2, m), 0.97 (2-Me, d; 6.9), 3.23 (3, dd; −13.3 and 7.7), 3.14 (3, dd; −13.3 and 6.3); ^{13}C NMR (CD_3OD) unit δ (carbon position) A 126.4 (2), 143.1 (3), 73.9 (5), 13.9 (6-Me), 66.1 (7), 60.5 (8), 139.2 (9), 126.6 (10/14), 129.5 (11/13), 129.1 (12); B 56.4 (2), 131.6 (4), 131.9 (5), 56.6 (7-OMe), 113.5 (8), 129.8 (9); C 26.4 (1-NMe), 15.6 (2-Me) with unassigned peaks at 43.7, 43.4, 41.6, 38.9 and 37.9.

N-Methylamide of leucic acid (**45**): ^{1}H NMR (CD_3OD) δ 4.01 (2, dd; 9.7, 3.6), 2.75 (NMe, s), 1.83 (4, m), 1.53 (3, m), 1.45 (3′, m), 0.94 (5, d; 6.7), 0.93 (4-Me, d; 6.7).

Effect of Cryptophycin-1 on Chromatin Condensation. Human ovarian SKOV3 cells were plated onto glass coverslips in Basal Media Eagle (BME) medium containing 10% fetal bovine serum (FBS) and 50 μg/mL of gentamycin sulfate. Following a 24−48 h attachment and growth period, the medium was removed and replaced with BME containing 0.5% FBS for 24 h. Following this 24 h period of serum deprivation,[44] **1** was added and the concentrations adjusted to 0, 50, 100, and 200 pM. After standing for 24 h, the cells were stained with acridine orange (1 mg/mL) for 10 min at 37 °C and then viewed using a Zeiss Axioplan fluorescence microscope with optics for fluorescein. The condensed nuclei were counted in a minimum of seven microscopic fields. The results are shown in Table 3.

Cryptophycin-1-Induced DNA Fragmentation in L1210 Cells. L1210 cells were treated with vehicle, 200 pM **1**, or 1 μM dexamethazone for 4 h at 37 °C. The DNA was extracted using an Applied Biosystems Genepure 341 nucleic acid purification system based on organic extraction and alcohol extraction. The purified DNA was separated by agarose electrophoresis using 2% Nusieve (3:1) agarose, stained with ethidium bromide and photographed under ultraviolet light.

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Supporting Information Available: Tables of ^{1}H NMR data of **1** and **3** in DMSO-d_6, $CDCl_3$, and CD_2Cl_2; 500 MHz ^{1}H NMR spectra of **8**−**25** in $CDCl_3$; tables of bond distances, bond angles, anisotropic displacement coefficients (Å^2), atomic coordinates and equivalent isotropic displacement coefficients, and H-atom coordinates and equivalent isotropic displacement coefficients for **3** (26 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from Internet; see any current masthead page for ordering information and Internet access instructions.

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(44) This serum deprivation step synchronizes the cultures into a nonproliferative phase and provides essentially no chromatin condensation in the control cultures. This is critical because the antimicrotubule actions of cryptophycin cause interphase arrest and accompanying chromatin condensation in proliferating cultures.[4] Thus, serum deprivation prevents cell cycle progression so that chromatin condensation is not due to the cell cycle arrest, but rather fragmentation of DNA, a characteristic of apoptosis.