Three New Cryptophycins from *Nostoc* sp. GSV 224

Gottumukkala V. Subbaraju, Trimurtulu Golakoti, Gregory M. L. Patterson, and Richard E. Moore*

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

Received October 25, 1996[⊗]

Cryptophycin 46 (**2**), -175 (**3**), and -176 (**4**) have been identified as three new trace constituents of *Nostoc* sp. GSV 224. Cryptophycin-46 is an epimer of cryptophycin-3 (**5**) and to date is the only naturally occurring analogue having the *S* configuration at C-10 (C-2 in Unit B). Cryptophycins-175 and -176 also differ in unit B where **3** is the *O*-methyl analogue of cryptophycin-45 (**6**) and **4** is the *O*-desmethyl analogue of cryptophycin-21 (**8**). The relative and absolute stereochemistries of the three new analogues have been related to known cryptophycins by synthesis.

Cryptophycins are potent antitumor and antifungal peptolides that are found in the blue-green alga (cyanobacterium) *Nostoc* sp. GSV 224 (Nostocaceae).[1,2] The major naturally occurring representative of this class of cyclic depsipeptides, cryptophycin-1 (**1**), shows excellent activity against a broad spectrum of solid tumors, including drug-resistant ones, implanted in mice.[1,3] In addition to **1**, 21 other analogues have been isolated from the alga.[2] We report here the isolation and identification of three more cryptophycins (**2**−**4**) as trace constituents of GSV 224.

The three new analogues were isolated by extracting the lyophilized alga with 5:1 $CH_3CN-CH_2Cl_2$ and fractionating the concentrated extract by reversed-phase flash chromatography with mixtures of H_2O-CH_3CN as previously described.[2] All of the cryptophycins in the alga 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 (Figure 1). The subfraction eluting with the longest retention time, almost double the one for **1**, consisted of essentially one analogue, cryptophycin-46. The subfractions having retention times that were 0.59−0.75 and 0.45 the one for **1** contained cryptophycins-175 and -176, respectively. Repeated chromatography of these subfractions by normal-phase and/or reversed-phase HPLC led to the pure analogues. The gross structures and stereochemistries of **2**−**4** were established in a straightforward manner using a combination of spectral and chemical (total or semi-synthesis) techniques.

Cryptophycin-46 (**2**) had the molecular formula $C_{35}H_{43}ClN_2O_7$ based on its HREIMS. Its ^{1}H-NMR spectrum was similar to that of cryptophycin-3 (**5**), but the chemical shifts of several protons were different, particularly those for H-2 and H-3 in unit A and H_2-3 in unit C. The numbering system used for each of the two hydroxy acid and two amino acid units of the cryptophycins is shown in Figure 2. The NMR differences suggested that unit B in **2** had the 2*S* configuration and that cryptophycin-46 was 10-epicryptophycin-3.[4] The proposed structure was rigorously established by comparing the physical (optical rotations, HPLC retention times) and spectral (EIMS and ^{1}H NMR) data for natural and synthetic **2**.[4]

Cryptophycin-175 (**3**) had the molecular formula $C_{35}H_{42}Cl_2N_2O_7$ based on HREIMS. Its ^{1}H-NMR spectrum bore a close resemblance to that of cryptophycin-45 (**6**), but the signal for the phenolic hydroxyl was missing, and an *O*-methyl signal could be seen at 3.86 ppm. Furthermore, the mass spectrum of **3** exhibited characteristic fragment ions[5] at *m*/*z* 446/448/450 (ion a), 314/316 (ion b), 229/231 (ion d), and 227 (ion c), which supported the presence of two chlorines and a methoxyl group on the aromatic ring of unit B. Conversion of cryptophycin-31 (**7**) to **3** in two steps, that is, (a) opening

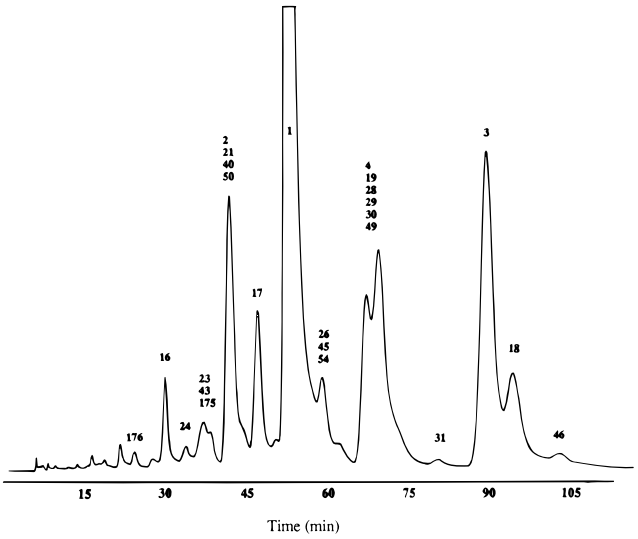


Figure 1. HPLC profile of the cryptophycin fraction from flash chromatography on an Econosil C18 column (25 cm × 22 mm, 10 μ) with 65:35 MeCN−H_2O (flow rate 6 mL/min, UV detection at 254 nm).

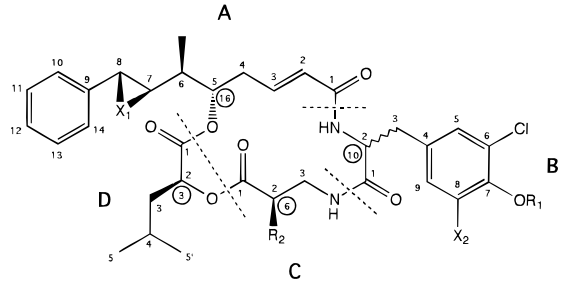


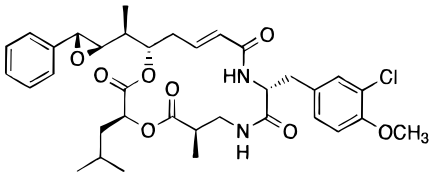
Figure 2. Structure and absolute stereochemistry of naturally occurring cryptophycins (X_1 = O or π-bond; X_2 = H or Cl; R_1/R_2 = H and/or Me). The numbering system for the two hydroxy acid units A and D and the two amino acid units B and C is used for the representation of the ^{1}H- and ^{13}C-NMR data. Numbers enclosed in circles refer to the IUPAC numbering system found in Chemical Abstracts.

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1997.

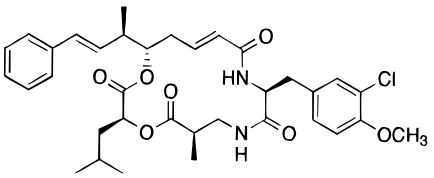
S0163-3864(96)00700-8 CCC: $14.00

the epoxide ring to a bromohydrin with HBr−AcOH followed by (b) reducing the bromohydrin to the alkene with Zn−AcOH, established that the two compounds had the same absolute stereochemistry.

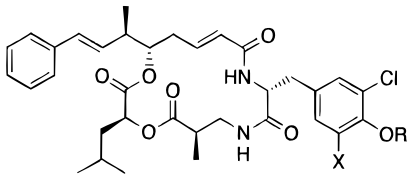
Cryptophycin-176 (**4**) had the molecular composition $C_{33}H_{39}ClN_2O_8$ based on HREIMS. Its ^{1}H-NMR spectrum was very similar to that of cryptophycin-21 (**8**), but the *O*-methyl signal was missing, and a phenolic hydroxyl signal was present at 9.93 ppm**.** Moreover, the ^{13}C-NMR spectrum of **4** also lacked an *O*-methyl signal and displayed an upfield shift of the signal for C-7 in unit B from 153.9 ppm for **8** to 151.4 ppm for **4**, which was consistent for replacement of the *O*-methyl group by a phenolic OH. Conversion of **8** into **4** by the following sequence of transformations—(a) opening of the epoxide ring to a mixture of chlorohydrins with TMSCl, (b) demethylation to the phenol with BBr_3−SMe_2, and (c) regeneration of the epoxide ring with K_2CO_3—confirmed the structure and further established that **4** had the same absolute stereochemistry as **8**.



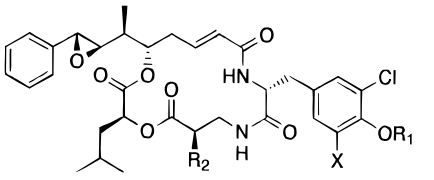
cryptophycin-1 1



cryptophycin-46 2



cryptophycin-175	**3**	$R = CH_3$	$X = Cl$	
cryptophycin-3	**5**	$R = CH_3$	$X = H$	
cryptophycin-45	**6**	$R = H$	$X = Cl$	



cryptophycin-176	**4**	$R_1 = H$	$X = H$	$R_2 = H$
cryptophycin-31	**7**	$R_1 = CH_3$	$X = Cl$	$R_2 = CH_3$
cryptophycin-21	**8**	$R_1 = CH_3$	$X = H$	$R_2 = H$
cryptophycin-16	**9**	$R_1 = H$	$X = H$	$R_2 = CH_3$
cryptophycin-23	**10**	$R_1 = H$	$X = Cl$	$R_2 = CH_3$

The three new analogs are weaker cytotoxins than **1** or **5** against the human tumor cell lines KB (nasopharyngeal), LoVo (colorectal), and SKOV3 (ovarian) for the following reasons: (a) Cryptophycin-46 (**2**) is weaker than **5** because the configuration of unit B has to be *R* for maximum activity. Changing it to *S*, as it is in **2**, results in a several-hundred-fold loss in cytotoxicity. Cryptophycin-46 (**2**) shows IC_{50} values ranging from 750 to 1100 nM compared with 1.9−4.4 nM for **5**.[2] (b) Cryptophycin-175 (**3**) is weaker than **5** because addition of a second chlorine to the aromatic ring in unit B results in a 10-fold to hundredfold loss of cytotoxicity.[2] Cryptophycin-175 (**3**) shows IC_{50} values around 100 nM compared with 1.9−4.4 nM for **5**. (c) Cryptophycin-176 (**4**) is weaker than **1** because removal of the *O*-methyl group in unit B results in at least a 10-fold loss of cytotoxicity.[2] Cryptophycin-176 (**4**) shows IC_{50} values in the 1.3−1.6 nM region, compared with 0.0092−0.02 nM for **1**.[2] Removal of the methyl group in unit C, however, does not affect the cytotoxicity significantly. The potency of **4** is only a little less that of cryptophycin-16 (**9**, IC_{50} 0.3−0.6 nM).[2]

Experimental Section

Spectral Analysis. NMR spectra were determined on an 11.75-T (GN-OMEGA) instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H chemical shifts are referenced in DMSO-d_6 and $CDCl_3$ to residual DMSO-d_5, (2.49 ppm) and $CHCl_3$ (7.24 ppm); ^{13}C chemical shifts are referenced to the solvent (DMSO-d_6, 39.5; $CDCl_3$, 77.0 ppm). All ^{1}H and ^{13}C assignments are based on detailed COSY, HMQC, and HMBC analyses where one-bond ^{1}H-^{13}C connectivities have been determined by HMQC and two- and three-bond ^{1}H−^{13}C connectivities have been determined by HMBC. Mass spectra, including HRMS measurements, were determined in the EI mode on a VG-70SE instrument. UV and IR spectra and optical rotations were measured on a Beckmann DU-7000 UV spectrometer, Perkin-Elmer 1600 FTIR, and a JASCO DIP-370 digital polarimeter, respectively.

Isolation. Lyophilized *Nostoc* sp. GSV 224 (250 g) was extracted with 5:1 MeCN−CH_2Cl_2 and the extract subjected to reversed-phase flash column chromatography and HPLC (Econosil C18) as previously described.[2] The fraction that eluted from the Econosil C18 column at t_R 103.5 min was subjected to HPLC on an Econosil silica column (250 × 10 mm, 5μ, 1:1 EtOAc−hexanes, 3 mL/min) to give cryptophycin-46 (**2**, t_R 31 min, 0.1 mg). The fraction that eluted from the Econosil C18 column at t_R 31−40 min was subjected to HPLC twice on the Econosil silica column, using 55:45 EtOAc−hexanes for the first run and 4:6 EtOAc−CH_2Cl_2 for the second run, to give cryptophycin-175 (**3**, t_R 12.2 min for first HPLC and t_R 9 min for second HPLC, 0.1 mg). The material that was eluted from the Econosil C18 column at t_R 24 min was subjected to HPLC on the Econosil silica column with 6:4 EtOAc−hexanes to give cryptophycin-176 (**4**, t_R 54 min, 0.1 mg). Additional **4** (0.1 mg) was isolated from the fraction (0.3 g) that was eluted with 1:1 MeCN−H_2O from the original reversed-phase flash column[2] by subjecting it to reversed-phase HPLC on the Econosil C-18 with 65:35 MeCN−H_2O (6 mL/min) and further fractionating the material eluting at t_R 24 min to normal-phase HPLC on the Econosil silica column as described above.

Cryptophycin-46 (2): $[\alpha]_D$ −62.1° ($CHCl_3$, *c* 0.66); EIMS[5] *m/z* (rel int, assignment) 638/640 (9.0/2.6, M^+), 412/414 (62.9/24.5, ion a), 280/282 (11.8/3.5, ion b), 227

Table 1. ^{1}H-NMR Data for Cryptophycins-46 (**2**), -175 (**3**), and -176 (**4**)

		cryptophycin-		
	position	46 (**2**)[a]	175 (**3**)[a]	176 (**4**)[b]
A	2	5.88 (d, 16.0)	5.81 (d, 15.4)	5.76 (d, 14.8)
	3	6.43 (m)	6.66 (ddd, 15.3, 9.5, 5.8)	6.42 (ddd, 15.0, 11.1, 3.9)
	4	2.52 (m)	2.54 (m)	2.60 (m)
	4′	2.40 (m)	2.39 (m)	2.26 (m)
	5	4.98 (m)	4.97 (m)	5.11 (m)
	6	2.58(m)	2.56 (m)	1.81 (m)
	6-Me	1.15 (d, 7.0)	1.14 (d, 6.7)	1.04 (d, 6.6)
	7	6.00 (dd, 15.8, 8.8)	6.01 (dd, 15.8, 8.9)	2.90 (d, 7.7)
	8	6.43 (d, 15.8)	6.42 (d, 15.8)	3.88 (br s)
	10/11/13/14	7.21−7.34 (m)	7.30−7.34 (m)	7.29−7.39 (m)
	12	7.21−7.34 (m)	7.22 (m)	7.29−7.39 (m)
B	2	4.82 (m)	4.82 (m)	4.25 (m)
	2-NH	5.66 (d, 9.2)	5.85 (d, 8.9)	8.23 (d, 8.5)
	3	3.10 (dd, 14.5, 7.2)	3.01 (dd, 14.4, 7.2)	2.67 (m)
	3′	3.14 (dd, 14.4, 5.4)	3.14 (dd, 14.3, 5.6)	2.91 (dd, 14.6, 3.8)
	5	7.22 (d, 2.0)	7.17 (s)	7.15 (br s)
	7-OMe	3.87 (s)	3.86 (s)	
	7-OH			9.93 (s)
	8	6.85 (d, 8.3)		6.83 (d, 8.2)
	9	7.09 (dd, 8.5, 2.1)	7.17 (s)	6.96 (br d, 8.8)
C	2	2.68 (m)	2.73 (m)	2.62 (m)
	2′			2.32 (m)
	2-Me	1.17 (d, 7.2)	1.21 (d, 7.2)	
	3	3.45 (m)	3.19 (ddd, 13.6, 6.8, 6.8)	3.21 (m)
	3′	3.45 (m)	3.58 (m)	3.21 (m)
	3-NH	6.84 (m)	6.87 (br t, 5.9)	7.21 (br t, 5.5)
D	2	4.92 (dd, 10.0, 3.4)	4.82 (dd, 10.1, 3.5)	4.95 (dd, 10.3, 4.3)
	3	1.40 (m)	1.37 (m)	1.21 (m)
	3′	1.61 (m)	1.65 (m)	1.44−1.56 (m)
	4	1.61 (m)	1.65 (m)	1.44−1.56 (m)
	4-Me	0.79 (d, 6.6)	0.77 (d, 6.5)	0.76 (d, 6.3)
	5	0.72 (d, 6.6)	0.72 (d, 6.5)	0.74 (d, 6.6)

[a] $CDCl_3$. [b] DMSO-d_6.

(66.5, ion c), 195/197 (57.8/16.2, ion d), 168 (65.1), 91 (100); HREIMS *m/z* 638.2767 (calcd for $C_{35}H_{43}ClN_2O_7$, −0.8 mmu error). ^{1}H NMR data, see Table 1.

Cryptophycin-175 (3): $[\alpha]_D$ +32.8° ($CHCl_3$, *c* 0.81); UV (MeOH) λ_{max} (ϵ) 208 (84 800), 210 (78 700), 228 sh (32 100), 249 (27 300), 284 nm (3041); IR (neat) ν_{max} 2961, 1747, 1727, 1682, 1538, 1481, 1001, 971, 802, 749, and 694 cm^{-1}; EIMS[5] *m/z* (rel int, assignment) 672/674/678 (2.3/1.3/0.3, M^+), 446/448/450 (11.7/8.4/2.3, ion a), 314/316 (6.1/3.0, ion b), 281/283 (17.2/3.1), 229/231 (13.2/7.9, ion d), 227 (100, ion c), 183 (19.9), 131 (38.6), 91 (35.8); HREIMS *m/z* 672.2356 (calcd for $C_{35}H_{42}Cl_2N_2O_7$, 1.3 mmu error). ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2.

Cryptophycin-176 (4): $[\alpha]_D$ +40.5° (MeOH, *c* 0.38); UV (MeOH) λ_{max} (ϵ) 206 (39 900), 220 sh (26 600), 282 nm (2,00); IR (neat) ν_{max} 3417 (br), 1732, 1694, 1668, 1652, 1644, 1634, 1538, 1504, 1372, 1173, and 694 cm^{-1}; EIMS[5] *m/z* (rel int, assignment) 626/628 (17.0/10.4, M^+), 384 (8, ion a), 251 (16), 227 (11, ion c), 105 (20), 91 (100); HREIMS *m/z* 626.2390 (calcd for $C_{33}H_{39}ClN_2O_8$, 0.5 mmu error). ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2.

Conversion of 7 into 3. Cryptophycin-31 (**7**, 20 mg), produced from methylation of cryptophycin-23 (**10**) as previously described,[2] in DME (1 mL) was treated with HBr in HOAc (30 wt % solution, 10 μL) at −78 °C. The reaction mixture was allowed to warm to and stand at room temperature overnight. The excess acid was neutralized with solid K_2CO_3 and then the mixture was filtered through nylon filters. The filtrate was evaporated, and the crude bromohydrin in CH_2Cl_2 (1 mL) was treated with Zn (15 mg) and HOAc (0.1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, stirred for 6 h, and then passed through a nylon filter. The solvent was evaporated, and the

Table 2. ^{13}C-NMR Data for Cryptophycins-175 (**3**) and -176 (**4**)

		cryptophycin-	
	carbon	175 (**3**)[a]	176 (**4**)[b]
A	1	165.6	164.7
	2	125.3	125.5
	3	141.4	139.5
	4	36.5	32.5
	5	77.5	75.7
	6	42.3	c
	6-Me	17.4	13.6
	7	131.9	62.7
	8	130.0	58.1
	9	136.7	137.2
	10/14	126.2	125.9
	11/13	128.6	128.5
	12	127.6	128.3
B	1	171.0	170.4
	2	53.3	55.0
	3	35.2	34.6
	4	134.7	129.8
	5	129.7	130.0
	6	129.2	119.1
	7	151.1	151.4
	7-OMe	60.7	
	8	129.2	116.4
	9	129.7	128.2
C	1	175.3	171.6
	2	38.3	36.4
	2-Me	14.0	
	3	41.5	33.2
D	1	170.7	169.9
	2	71.5	70.0
	3	39.5	c
	4	24.5	23.7
	4-Me	22.7	22.4
	5	21.2	21.1

[a] $CDCl_3$. [b] DMSO-d_6. [c] Hidden by solvent signals.

residue was subjected to reversed-phase HPLC on an Econosil C18 column (250 × 22 mm, 10 μ, 6 mL/min) with 7:3 MeCN−H_2O to give cryptophycin-175 (**3**, 12 mg, t_R: 83 min) in a 60% overall yield. Semisynthetic and naturally occurring **3** had identical ^{1}H- and ^{13}C-NMR spectra.

Conversion of 8 into 4. Cryptophycin-21 (**8**, 30 mg) in CH_2Cl_2 (5 mL) was treated with chlorotrimethylsilane (60 μL) at −78 °C. and allowed the reaction mixture to warmup to room temperature. After 2h, the solvent was removed under N_2, and the residue was chromatographed over a small silica column (0.5 g) using CH_2Cl_2 and 3:2 CH_2Cl_2−EtOAc successively for elution. The latter fraction gave a 2:1 mixture of chlorohydrins (30 mg), which was dissolved in DCE (5 mL) and treated with BBr_3−Me_2S (160 mg) at room temperature and then under reflux for 20 h. The reaction mixture was quenched with H_2O and evaporated *in vacuo*. The residue was chromatographed on a reversed-phase C18 column (12 × 1 cm) with mixtures of MeCN−H_2O to give phenolic chlorohydrins (14.6 mg) and unreacted starting material (7.3 mg). The phenolic chlorohydrins fraction (10 mg) in Me_2CO was treated with K_2CO_3 (20 mg) at room temperature and heated at 50−60 °C for 20 h. The reaction mixture was filtered through a nylon filter to remove K_2CO_3 and evaporated. The residue was subjected to reversed-phase HPLC on an Econosil C18 column (250 × 22 mm, 10 μ, 6 mL/min) with 65:35 MeCN−H_2O to give cryptophycin-176 (**4**, 6 mg, t_R: 22 min) in a 30% overall yield. Semisynthetic and naturally occurring **4** had identical ^{1}H- and ^{13}C-NMR spectra.

Acknowledgment. This research was supported by Grant No. CA12623 from the National Cancer Institute, Department of Health and Human Services.

References and Notes

(1) Trimurtulu, G.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994**, *116*, 4729−4737.
(2) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Husebo, T. L.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12 030−12 049.
(3) Moore, R. E.; Corbett, T. H.; Patterson, G. M. L.; Valeriote, F. A. *Curr. Pharm. Design* **1996**, *2*, 317−330.
(4) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479−2490.
(5) For an explanation of ions a, b, c, and d, refer to Scheme 1 in our first publication.[1]

NP960700A