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Volutamides A-E, Halogenated Alkaloids with Antifeedant Properties from the Atlantic Bryozoan *Amathia convoluta*

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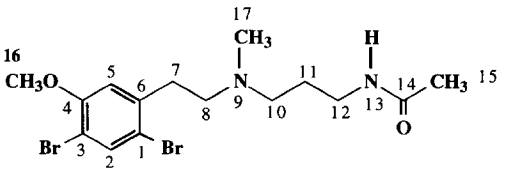
Abstract: Volutamides A-E (**1-5**), halogenated alkaloids of amino acid origin, have been isolated from the temperate Atlantic bryozoan *Amathia convoluta* Lamouroux. The structures of the new compounds were determined by spectral and chemical methods. The absolute stereochemistries of volutamides B and C were established by CD measurements, while the absolute stereochemistries of volutamides D and E could not be established with confidence. Several of the volutamides deterred feeding by potential predators and were toxic toward larvae of a co-occurring hydroid, suggesting that these metabolites form the basis of an effective chemical defense. Copyright © 1996 Elsevier Science Ltd

An increasing number of empirical studies confirm that structurally diverse secondary metabolites of marine plants and animals can act as defenses against consumers.[2] Most commonly, secondary compounds of tropical macroalgae and invertebrates have been reported to deter potential consumers. Much less is known, however, about the chemical defenses of temperate marine organisms. Along the temperate Atlantic coast of North Carolina (USA), the bryozoan *Amathia convoluta* Lamouroux is a conspicuous member of shallow hard-bottom communities. Preliminary ecological studies showed that fish and urchins typically do not consume *A. convoluta*, and that the crude extract of this bryozoan inhibits feeding by the abundant omnivorous pinfish *Lagodon rhomboides*.[3] In this paper, we report that North Carolina *A. convoluta* contains five new halogenated alkaloids, the volutamides A-E (**1-5**), several of which deter fish and urchin feeding. The volutamides are related biosynthetically to the amathamides, proline based alkaloids from the Australian bryozoans *A. wilsoni* and *A. pinnata*,[4-6] and to a lesser extent to the convolutamides, convolutamydines, and convolutamines, metabolites recently reported from a Florida collection of *A. convoluta*.[7-9] *A. convoluta* from Tasmania is reported to contain proline-based alkaloids[10] rather than the leucine and tyrosine derivatives reported here.

Structures of Volutamides A-E



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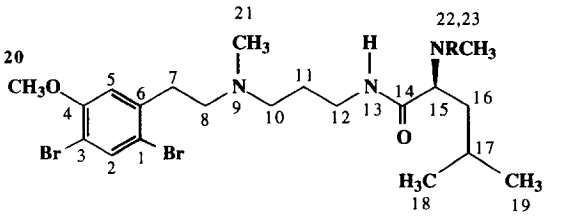
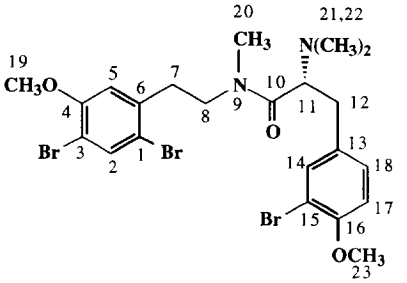
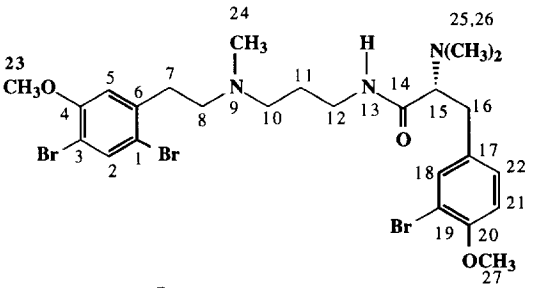


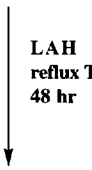
2, VOLUTAMIDE B, R = H
3, VOLUTAMIDE C, R = CH_3

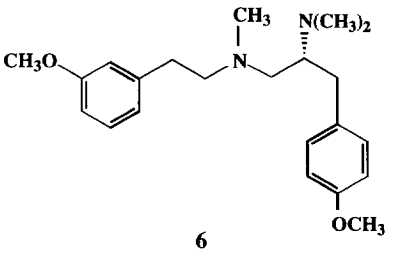


4, VOLUTAMIDE D



5, VOLUTAMIDE E





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Amathia convoluta was collected by hand using SCUBA and immediately frozen. After freeze-drying, the animal was pulverized and exhaustively extracted with dichloromethane/methanol (1/1). When the extract was incorporated into a squid-based food at natural concentrations, it reduced pinfish feeding by 93%.[3] After partitioning the crude extract between dichloromethane and water, we found that the water-soluble portion had no effect on fish feeding using the same assay. Isolation of the deterrent metabolites, along with several related molecules, from the dichloromethane soluble material was then achieved by bioassay-guided fractionation (see experimental). The structure determinations of these metabolites are discussed below.

Volutamide A (**1**), the simplest metabolite isolated, was assigned the molecular formula $C_{15}H_{22}N_2O_2Br_2$ on the basis of its high resolution mass and NMR spectral data (Tables 1 and 2). The ^1H and ^{13}C NMR data for **1**, including DEPT and XHCORR ^{13}C measurements, showed the presence of five methylenes and a tetrasubstituted benzene ring. The methylenes were assigned by COSY analysis to spin-isolated ethylene and propylene units. The aromatic protons, which appeared as singlets at δ 7.68 and 6.78 in the ^1H NMR spectrum, were assigned in a *para* orientation. Proton and ^{13}C data for the aromatic and ethylene portions of volutamide A were strikingly similar to those reported for 2-(2,4-dibromo-5-methoxyphenyl)ethylamine, and compounds containing similar features isolated from sponges.[11-12] The regiochemistry of this configuration was confirmed by n.O.e. measurements which placed the C-7, C-8 ethylene unit and methoxy group *ortho* to the δ 6.78 aromatic proton. Irradiation of the C-5 aromatic proton produced enhancements of the C-7 protons (δ 2.89, 2H) and enhanced the methoxy group, thus allowing the methoxy group to be positioned at C-4. The infrared spectrum of **1** showed

Table 1. ^1H NMR Spectral Assignments for Volutamides A-C and E (1-3, 5)*

H at C/N#	Volutamide A (**1**)	Volutamide B (**2**)	Volutamide C (**3**)	Volutamide E (**5**)
2	7.68 (1H, s)	7.67 (1H, s)	7.66 (1H, s)	7.70 (1H, s)
5	6.78 (1H, s)	6.80 (1H, s)	6.79 (1H, s)	6.80 (1H, s)
7	2.89 (2H, m)	2.88 (2H, m)	2.86 (2H, m)	2.90 (2H, m)
8	2.66 (2H, m)	2.66 (2H, m)	2.62 (2H, m)	2.61 (2H, m)
10	2.56 (2H, t, 6.5)	2.58 (2H, t, 6.5)	2.57 (2H, t, 6.5)	2.52 (2H, t, 6.5)
11	1.71 (2H, m)	1.74 (2H, m)	1.71 (2H, m)	1.86 (2H, m)
12	3.32 (2H, m)	3.33 (2H, m)	3.31 (2H, m)	3.26 (2H, m)
13	6.63 (1H, t, 8.5)	7.62 (1H, t, 8)	7.44 (1H, t, 8)	7.60 (1H, t, 6)
15	1.92 (3H, s)	2.90 (1H, dd, 13, 7)	2.86 (dd, 13, 6)	3.14 (1H, dd, 9, 4)
16	3.88 (3H, s)	1.56 (1H, m)	1.56 (1H, m)	2.80 (1H, m)
		1.36 (1H, m)	1.36 (1H, m)	3.07 (1H, m)
17	2.36 (3H, s)	1.70 (1H, m)	1.71 (1H, m)	--
18	--	0.94 (3H, d, 6)	0.94 (3H, d, 6)	7.41 (1H, d, 2)
19	--	0.92 (3H, d, 6)	0.92 (3H, d, 6)	--
20	--	3.84 (3H, s)	3.87 (3H, s)	--
21	--	2.38 (3H, bs)	2.35 (3H, s)	6.78 (1H, d, 8)
22	--	not obs.	2.27 (3H, s)	7.17 (1H, dd, 8, 2)
23		2.36 (3H, bs)	2.27 (3H, s)	3.88 (3H, s)
24	--	--	--	2.32 (3H, s)
25	--	--	--	2.32 (3H, s)
26	--	--	--	2.32 (3H, s)
27	--	--	--	3.83 (3H, s)

* Spectra were recorded at 360 MHz in $CDCl_3$. Chemical shifts, in δ units, were assigned by COSY analyses, by comparison with analogous metabolites, and by XHCORR and COLOC correlation methods.

Table 2. ^{13}C NMR Spectral Assignments for Volutamides A-C and E (1-3, 5)*

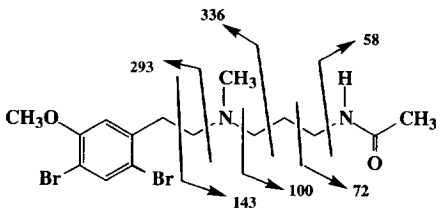
C#	Volutamide A (**1**)	Volutamide B (**2**)	Volutamide C (**3**)	Volutamide E (**5**)
1	110.1(C)[a]	110.3(C)[a]	109.8(C)[a]	111.2(C)[a]
2	136.2(CH)	135.5(CH)	135.9(CH)	136.0(CH)
3	114.8(C)[a]	114.0(C)[a]	114.7(C)[a]	114.7(C)[a]
4	155.2(C)	155.3(C)	155.2(C)	155.3(C)
5	113.6(CH)	113.8(CH)	113.7(CH)	113.8(CH)
6	139.4(C)	138.0(C)	139.7(C)	139.8(C)
7	33.7(CH_2)	32.1(CH_2)	33.7(CH_2)	32.0(CH_2)
8	57.2(CH_2)	55.8(CH_2)	57.2(CH_2)	56.9(CH_2)
10	56.1(CH_2)	53.8(CH_2)	55.3(CH_2)	55.0(CH_2)
11	25.8(CH_2)	25.3(CH_2)	26.7(CH_2)	26.2(CH_2)
12	39.0(CH_2)	36.9(CH_2)	37.7(CH_2)	37.8(CH_2)
14	170.0(C)	172.0(C)	173.1(C)	171.5(C)
15	23.3(CH_3)	62.1(CH)	67.4(CH)	71.0(CH)
16	56.4(CH_3)	40.6(CH_2)	36.9(CH_2)	56.9(CH_2)
17	41.7(CH_3)	24.8(CH)	25.7(CH)	133.2(C)
18	--	22.5(CH_3)	23.3(CH_3)	133.8(CH)
19	--	22.3(CH_3)	22.0(CH_3)	110.0(C)
20	--	56.3(CH_3)	56.3(CH_3)	154.1(C)
21	--	40.4(CH_3)	41.7(CH_3)	111.7(CH)
22	--	--	42.1(CH_3)	129.4(CH)
23	--	40.6(CH_3)	42.1(CH_3)	56.4(CH_3)
24	--	--	--	41.4(CH_3)
25	--	--	--	42.1(CH_3)
26	--	--	--	42.1(CH_3)
27	--	--	--	56.1(CH_3)

* Spectra recorded at 50 MHz in $CDCl_3$. Assignments were based upon XHCORR experiments and by comparison with literature standards. [a]Assignments within the vertical column may be interchanged.

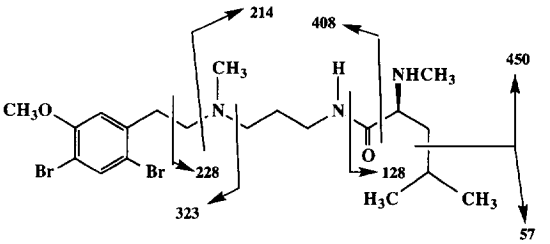
an intense carbonyl absorption band at 1644 cm^{-1} which, along with the ^{13}C-NMR signal at δ 170.0 (Table 2), indicated that volutamide A contained an amide. The presence of a 3 proton singlet at δ 1.92 in the ^1H NMR spectrum of **1** further indicated that the amide was an acetamide. COSY analysis showed that the amide proton was coupled to methylene protons (δ 3.32, 2H, m) which were part of the propylene unit. The remaining unassigned atoms in **1** were assigned, by ^1H NMR chemical shift considerations, as an N-methylamino functionality (δ 2.36, s, 3H), connecting the ethylene and propylene subunits. These assignments, and hence the complete structure of volutamide A, was confirmed by mass spectral analysis (Figure 1) which showed sequential

fragmentations consistent with this linear array.

Volutamide B (**2**), a viscous oil, was assigned the molecular formula $C_{20}H_{33}N_3O_2Br_2$ on the basis of high resolution mass and NMR spectral data (Tables 1 and 2). NMR data for **2** showed numerous similarities to volutamide A plus several new resonances. The same phenylethyl-N(CH_3)-propylene subunit of **1** was clearly present in **2** based on n.O.e. and COSY experiments. The major difference in the ^1H NMR spectrum of **2**



1



2

Figure 1. MS(EI) Fragmentations of Volutamide A (**1**) and Volutamide B (**2**)

compared to that of **1** was the lack of the acetamide group and the appearance of resonances assignable to an N-methyl leucine residue. COSY analysis of this amino acid residue showed two methyl doublets at δ 0.93 and 0.95 coupled to a methine proton at δ 1.7. This latter proton was also coupled to two vicinally-coupled methylene protons (δ 1.36 and 1.56), each of which coupled to the deshielded methine proton at δ 2.90. Analysis of the fragmentation pattern generated by ms-ms methods showed sequential fragmentation of the N-methyl leucine unit, thus confirming the structure of volutamide B (Figure 1).

Volutamide C (**3**) was isolated as a viscous oil which analyzed for $C_{21}H_{35}N_3O_2Br_2$ by high resolution mass spectral and NMR methods. The ^1H NMR spectrum of **3** was virtually identical to that of volutamide B, except that a 6 proton N-methyl signal (δ 2.27) was observed instead of the 3 proton N-methyl signal (δ 2.36) in **2**. Hence, **3** was assigned as the corresponding dimethyl analog of **2**.

Volutamide D (**4**) was determined to have the molecular formula $C_{22}H_{28}N_2O_3Br_3$ by analysis of its high resolution mass spectral and NMR spectral data. NMR analysis of **4**, however, was not as straightforward as for volutamides A-C due to the persistent doubling of many of the ^1H and ^{13}C NMR resonances (see experimental section). The IR spectrum of **4** showed a strong band at 1643 cm^{-1} which was assigned to an amide carbonyl. The presence of this amide linkage explained the complexity of the NMR spectrum assuming that two amide rotational isomers were present in roughly equal amounts. To test this hypothesis, we attempted to reduce the amide (in **4**) with lithium aluminum hydride. Volutamide D was refluxed with $LiAlH_4$ in tetrahydrofuran for 48 hours. A single recognizable reduction product, **6**, was obtained after purification of the reaction mixture. The reduction product **6** did not show carbonyl IR absorptions indicating reduction of the amide had occurred. This was also confirmed by the lack of a carbonyl resonance in the ^{13}C NMR spectrum of the compound. The molecular formula $C_{22}H_{33}N_2O_2$, established by high resolution FAB mass spectral methods and ^1H NMR data, indicated that $LiAlH_4$ reduction had eliminated all bromine atoms on the aromatic rings. The final structure assignment for **6** followed by comprehensive analysis of NMR and other spectral data (see experimental). The structure of the reduction product **6** gave considerable insight into the final structure assignment for volutamide D. But, the structure still could not be assigned with confidence.

Fortunately, volutamide D (**4**) crystallized from methanol yielding crystals suitable for single crystal X-ray analysis. The crystals obtained formed in the triclinic space group P1 with four (Z=4) independent molecules in the asymmetric unit. A drawing of one of the crystalline conformers is shown in Figure 2. All of the volutamide D molecules in the crystal had the same overall structure, but they differed in their amide conformations. Two had *trans* amide bonds and two had *cis*. The two with *trans* amide bonds differed through a rotation about the C10-N9 bond, while the two with *cis* amide bonds had a more complex rotational isomeric relationship involving rotation about several sigma bonds (see experimental). Assignment of the absolute configuration was attempted using the anomalous scattering method. All molecules in the crystal were found to possess the same absolute configuration. The configuration at C11 appeared to be **R**, as shown in Figure 2, which corresponds to a **D** amino acid. But because of the well-known insecurities of this method, we view this assignment as tentative.

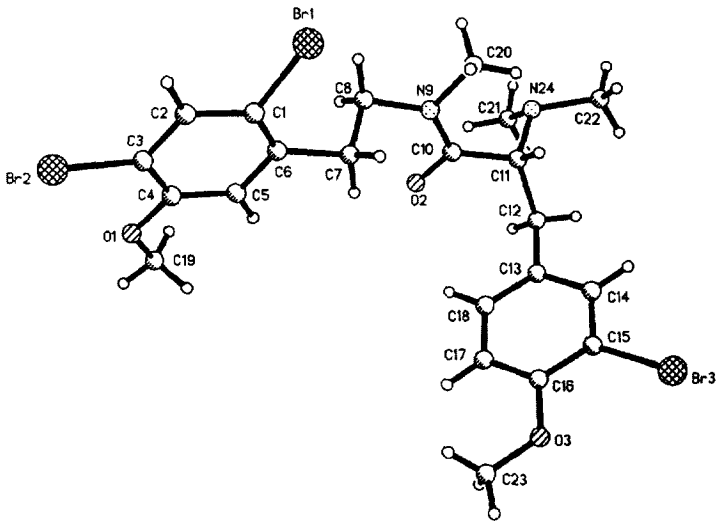


Figure 2. Stereostructure of one of the final X-ray structures of volutamide D (**4**).

Volutamide E (**5**), a viscous oil, was determined to have the molecular formula $C_{25}H_{35}N_3O_3Br_3$ by interpretation of its high resolution mass and NMR spectral data. Because all ^1H and ^{13}C NMR resonances assigned to the substituted phenylethyl-N(Me)-propyl-amide subunit of volutamides A-C also were observed for **5** (Tables 1 and 2), this subunit was clearly present in volutamide E. For the remaining atoms, $C_{11}H_{15}NOBr$, a 1, 2, 4-trisubstituted benzene ring was indicated by the coupling pattern of the aromatic protons (Table 1). The δ 6.78 doublet proton was placed adjacent to the methoxy substituent (δ 3.83) because of the upfield shift of this aromatic proton and in recognition of its 9% enhancement upon irradiation of the methoxy protons. Bromine was assigned to the other carbon adjacent to the methoxy group on the basis of the significant upfield shift of this carbon (δ 110). A methylene (at δ 56.9 in the ^{13}C NMR spectrum) was determined to be the third substituent on the aromatic ring. The methylene protons of **5** were further coupled to a methine proton at δ 3.14 (dd, J = 9, 4 Hz) which was assigned as the proton on the alpha carbon of an amino acid residue. The combined data for volutamide E showed that the identical 3'-bromo-4'-methoxyphenylalanine residue as in **4** was present in **5**.

To determine the absolute stereochemistry of the amino acid residues in volutamides B-E, we attempted to hydrolyze the amide linkages to isolate the free amino acids. Even though hydrolysis was performed with strong acids at high temperatures, the compounds (volutamides B-E) were found to be resistant to even the most rigorous

hydrolysis conditions. After refluxing volutamide D in concentrated HCl for 48 hours, for example, virtually all of the starting material was recovered. We next then turned to CD spectroscopy to assess the chirality of the amino acid portions of these metabolites. The use of optical rotatory dispersion and circular dichroism have long been employed for the assignment of absolute stereochemistry of α-amino acids. Free **L** amino acids typically give a positive cotton effect in the 210-240 nm range.[13] The CD spectra of volutamides B and C showed strong positive absorption maxima at 224 nm, thus leading to their assignment as **L** amino acid derivatives. This assignment was further supported by synthesis of the benzyl esters of L-tyrosine and **L**-leucine, each of which also gave similar, well defined positive maxima. The CD spectra for volutamides D and E, on the other hand, were weak and broadened. Although a weak negative absorption maximum was observed at 230 nm suggesting the **D** configuration, the data must be considered inconclusive. Thus, the absolute stereochemistries of **4** and **5** have not been rigorously shown.

Among the purified metabolites from *Amathia convoluta*, volutamide B reduced pinfish feeding a significant 51%, and volutamide C reduced urchin feeding by 63%.[14] The only previous report of antipredator metabolites among bryozoans are the tambjamines A-D isolated from *Sessibugula translucens* collected in the Gulf of California.[15] Other investigations have shown that byrozoans may possess secondary metabolites that are toxic to microorganisms and larvae of competing invertebrates; thus bryozoan secondary metabolites also may function to prevent fouling.[16,17] Similarly, we found that volutamides B and D were toxic toward larvae of the co-occurring hydroid *Eudendrium carneum* within 24h when confined to seawater containing 10 μg/mL of either of these metabolites. Groups of control larvae not exposed to these compounds settled and metamorphosed within 72h.

This chemical investigation established the structures of five novel halogenated alkaloids from the temperate bryozoan *A. convoluta*. On the basis of preliminary bioassays, some of the volutamides may serve as chemical defenses against diverse generalist predators and against fouling by killing larvae of competing invertebrates. The biotesting results we obtained are clearly conservative when one considers the likely additive antifeedant bioactivity one would likely observe from the combined volutamides as they occur in the animal.

It is curious that *A. convoluta* from Florida does not contain the volutamides, but instead a series of only distantly-related metabolites, including the bryostatins.[7-9, 18] Although no data has been presented to illustrate the antipredator effects of metabolites from the Florida collections, it seems likely that these metabolites may also function as a chemical defense.

Lastly, as part of another project, the volutamides were tested in the phorbol ester-induced mouse ear adema assay, a whole animal assay used to detect anti-inflammatory agents. In this assay, volutamide B (**2**) showed weak inibition (12%) at the standard screening dose of 50 μg/ear. Under the identical conditions, volutamide C (**3**) showed 85% reduction, volutamide D (**4**) 48% reduction and volutamide E (**5**) 62% reduction of inflammation.

ACKNOWLEDGMENTS

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EXPERIMENTAL

General. Infrared spectra were recorded on a Perkin-Elmer FT-IR model 1600 spectrophotometer. Ultraviolet spectra were obtained in methanol using a Perkin Elmer model Lambda 3B spectrophotometer. ^{1}H NMR and COSY spectra were recorded in $CDCl_3$ solutions at 360-MHz. ^{13}C NMR spectra, direct (XHCORR), and long-range (COLOC) carbon-proton correlation spectra were recorded on an IBM WP-200 SY (50 MHz) spectrometer. All NMR chemical shifts are reported with respect to Me_4Si. Mass measurements were supplied by the Midwest Center for Mass Spectroscopy, University of Nebraska, Lincoln, NE. Optical rotations were measured in MeOH on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell. CD measurements were performed on a Cary 61 spectropolarimeter with a 0.02 cm cell by signal-averaging 20 scans. All solvents used were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Isolation. *Amathia convoluta* was collected by hand using SCUBA at -5 to -7 m depth along a rock jetty in Morehead City, NC, in June 1990. The collection was immediately frozen. The freeze-dried animal was exhaustively extracted with dichloromethane/methanol 1:1 (v/v). The extract obtained was redissolved in methanol and partitioned against hexanes. The methanol fraction (32.1 g) was then dissolved in *n*-butanol and partitioned against water. The chemically-rich butanol fraction (12.3 g) was then fractionated by silica vacuum flash chromatography, eluting with various proportions of dichloromethane in methanol, into 14 fractions. Chemically similar flash column fractions were combined and further fractionated by Sephadex LH-20 size exclusion chromatography using 100% methanol as the eluting solvent. Final purification of the volutamides was accomplished by the HPLC methods as specified below.

Volutamide A (**1**). Volutamide A, an oil, was eluted in silica fraction 9 with 10% MeOH in CH_2Cl_2. Further purification by LH-20 separated impure **1** in fractions 13-18. Final purification of **1** by C-18 reversed-phase HPLC (8/2 methanol/0.1M ammonium acetate) yielded 52 mg (0.014 % dry weight) of the purified metabolite. Volutamide A showed: IR (film): 3440br, 2950, 1644, 1510, 1400, 1240 cm^{-1}; UV(MeOH): λ_{max} = 285 nm (ε 4200); 292 nm (ε 4100); HRFABMS: M$^+$+H = 423.0089 for $C_{15}H_{23}N_2O_2{}^{79}Br_2$; LREIMS: *m/z* (% base) = 407 (0.05), 336 (0.5), 293 (4), 236 (0.5), 212 (2), 143 (100), 100 (79), 58 (20).

Volutamide B (**2**). Volutamide B, an oil, was eluted in silica fractions 12-13 with 85% MeOH in CH_2Cl_2. Further LH-20 purification gave impure **2** in fractions 7-10. Final purification by C-18 reversed-phase HPLC using 8/2 methanol/0.1M ammonium acetate to yield 202 mg (0.054 % dry weight) of volutamide B. Volutamide B showed: IR (film): 3326br, 2924, 1651, 1470, 1369, 1251 cm^{-1}; UV(MeOH): λ_{max} = 287 nm (ε 1600); 292 nm (ε 1500); HRFABMS: M$^+$+H = 508.0998, $C_{20}H_{34}N_3O_2{}^{79}Br^{81}Br$; MSMS data: *m/z* = 450, 408, 323, 228, 214, 128, 57; $[\alpha]_D$ = 0° (c 0.053, MeOH); CD: mean residual elipticity = +548 deg*cm^2*dmole^{-1} at 224 nm.

Volutamide C (**3**). Volutamide C, an oil, was purified from the same silica and LH-20 fractions as described above for **2** to yield 78 mg (0.021% dry weight) of **3**. Volutamide C showed: $[\alpha]_D$ = 0° (c 0.032, MeOH); IR (film): 3306br, 2952, 2866, 2787, 1652, 1582, 1518, 1471, 1369, 1250 cm^{-1}; UV(MeOH):λ_{max} = 285 nm (ε 2100); 293 nm (ε 1900); CD: mean residual elipticity = +750 deg*cm^2*dmole^{-1} at 224 nm; HRFABMS: M$^+$+H = 522.1152, $C_{21}H_{36}N_3O_2{}^{79}Br^{81}Br$; LRFABMS: M$^+$+H *m/z* = 522, *m/z* = 362, 242, 154, 114.

Volutamide D (**4**) Volutamide D was eluted in silica fraction 4 with 15% MeOH in CH_2Cl_2. Further LH-20 purification gave impure D in fractions 10-19. Final purification by C-18 reversed-phase HPLC, using 9/1 methanol/0.1 M $NH_4CH_3CO_2$ as the eluting solvent, gave 130 mg **4** (0.040 % dry weight). Volutamide D, as the amide rotamer mixture, showed: $[\alpha]_D$ = -12.9° (c 0.010, MeOH); ^{13}C NMR (MeOH-d_4): δ 177 br (Ac) 172.4, 171.8, 156.9, 156.4, 156.1, 139.9, 139.5, 137.1, 137.0, 135.3, 132.9, 131.8, 131.0, 130.7, 115.5, 115.3, 113.2, 112.3, 111.6, 111.2, 66.8, 66.6, 57.1, 56.9, 56.8, 42.1, 41.9, 36.2, 36.0, 34.6, 34.4, 33.5, 31.9, 22.4; ^{1}H NMR (MeOH-d_4): δ 7.6, 7.4, 7.2, 7.1, 7.0, 6.9, 6.8, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.2, 3.0, 2.9, 2.7, 2.5, 2.4, 1.7 (all bands broadened or twined); IR (film): 3462br, 2937, 2833, 2784, 1704, 1643, 1582, 1561, 1495, 1403, 1371 cm^{-1}; UV(MeOH): λ_{max} = 287 nm, (ε 3500); HRFABMS: M$^+$+H = 606.9612, $C_{22}H_{28}N_2O_3{}^{79}Br_2{}^{81}Br$; LRFABMS: M$^+$+H *m/z* = 607, *m/z* = 407, 293, 258, 256, 199.

Volutamide E (**5**) Volutamide E, a colorless oil, was eluted in silica fractions 10-11 with 12% MeOH in CH_2Cl_2. Further LH-20 purification gave impure E in fractions 6-10. Final purification by C-18 reversed-phase HPLC using 8/2 methanol/0.1M ammonium acetate yielded 236 mg of **5** (0.064% dry weight). Volutamide E showed:

$[\alpha]_D$ = -15.8° (c 0.014, MeOH); IR (film): 3420br, 2955, 1670, 1550, 1510, 1490, 1370 cm^{-1}; UV(MeOH): λ_{max} = 287 nm (ε 3800); HRFABMS: M^++H = 662.0345, $C_{25}H_{35}N_3O_3Br_3$.

Reduction Product **6**. Volutamide D (**4**) (32 mg) was treated with excess lithium aluminum hydride (45 mg) in dry tetrahydrofuran (50 ml) and refluxed for 48 hours. The solution was cooled and slowly quenched with cold water. The solution was then concentrated under vacuum and additional water and chloroform were added. The chloroform layer was removed and the water re-extracted twice with chloroform. The combined chloroform fractions were dried with anhyd. magnesium sulfate, filtered, and reduced under vacuum to yield a crude reaction mixture (12 mg). The crude product was purified by C-18 reversed-phase HPLC to give the highly reduced compound **6** (4 mg, 21% yield) as a colorless semi-solid. Reduction product **6** showed: $[\alpha]_D$ = -11°; ^{13}C NMR ($CDCl_3$): δ 159.6 (C), 157.7 (C), 142.3 (C), 132.8 (C), 129.9 (2xCH), 129.3 (CH), 121.1 (CH), 114.5 (CH), 113.7 (2xCH), 111.1 (CH), 63.8 (CH), 59.8 (CH2), 56.9 (CH2), 55.2 (CH3), 55.1 (CH3), 42.6 (CH3), 40.6 (2xCH3), 33.2 (CH2), 32.4 (CH2).; ^1H NMR ($CDCl_3$): δ 2.22 (s, 3H), 2.35 (s, 6H), 2.58 (m, 2H), 2.65 (m, 1H), 2.85 (m, 2H), 3.78 (s, 6H), 6.67 (dd, 1H, 2,2), 6.71 (dd, 1H, 7.3, 2), 6.82 (d, 2H, 8.5), 7.08 (d, 2H, 8.5); IR (film): 2934, 2833, 2776, 1610, 1584, 1511, 1488, 1454, 1299, 1246, 1176 cm^{-1}; UV(MeOH): λ_{max} = 282 nm (ε 3240), 287 nm (ε 3160); HRFABMS: M^++H = 357.2538, $C_{22}H_{33}N_2O_2$; LRFABMS: M^++H *m/z* = 357, *m/z* = 312, 178, 136, 121.

Hydroid Larvae Settling Bioassay. Larvae of the hydroid *Eudendrium carneum* were acquired from reproductive adults (collected in North Carolina) by carefully removing the individual larvae which are spontaneously spawned under laboratory conditions (25°C in seawater). Twenty of the individual orange-colored larvae were placed in small petri dishes with 10 ml of seawater, and test compounds (volutamides B and D) were added in ethanol (0.1%) to a final concentration of 10 μg/ml. Controls were performed using 20 identical larvae and transfer solvent only. The larvae were monitored for 48 hr, during which time control larvae underwent settling and metamorphosis into single polyps. Larvae exposed to volutamides B and D at 10μg/ml did not settle nor develop during the time course of the experiment. The results of the experiment were analyzed by the Fisher exact test, $P < 0.001$.

Single crystal X-ray diffraction analysis of volutamide D (**4**). Single crystals were grown from methanol, and a block with approximate dimensions 0.3 x 0.3 x 0.6 mm was chosen for all subsequent experiments. Preliminary diffraction photographs displayed only triclinic symmetry. Accurate cell constants of a = 11.824(2), b = 14.284(3), c = 15.423(4) Å, α = 88.39(2)°, β = 69.65(2)°, and γ = 81.70(2)° were obtained from a least-squares fit of twenty-five reflections with $30° \leq 2\theta \leq 45°$. Given a molecular formula of $C_{22}H_{27}Br_3N_2O_3$ and a significant optical rotation, the most plausible assignment of space group was P1 with Z = 4. Slightly more than a hemisphere of data was collected at -17 °C using θ:2θ scans and graphite monochromated CuKα radiation (1.54178 Å). No absorption of the reflections were judged observed ($|F_O| \geq 3\sigma(F_O)$). A phasing model was found using SHELXTL and several cycles of tangent formula recycling generated a map containing all of the nonhydrogen atoms. Least-squares refinements were not terribly stable, and a few atoms in molecules two and four had nonpositive definite thermal parameters. There was clearly some disorder in the C7, C8, C12 and N9 region of molecule two, and geometrical constraints were introduced. The final agreement factor was R = 0.086 for the observed data. An attempt to define the absolute configuration by the η-method led to η = 0.74 (11)- a value, while suggestive of a **D** configuration, must be considered inconclusive due to the known insecurities in this method. Interestingly, the four molecules within the unit cell each possessed an entirely different molecular conformation. Two possessed *trans* and two *cis* amide bonds about the C10-N9 bond. Descriptions of the precise conformations of all of these molecules are found in the crystallographic data which have been deposited with the Cambridge Crystallographic Data Centre.

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