Amathaspiramides A−F, Novel Brominated Alkaloids from the Marine Bryozoan *Amathia wilsoni*

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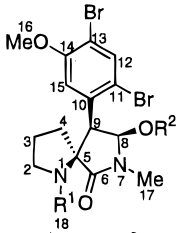
A series of six new dibrominated alkaloids, amathaspiramides A−F (**1**−**6**), have been isolated from a New Zealand collection of the marine bryozoan *Amathia wilsoni*. An X-ray crystallographic structural determination of amathaspiramide F (**6**) revealed it to be an epimer of amathaspiramide C (**3**). These data, in addition to the results of NOESY NMR experiments were used to infer the absolute configuration of the amathaspiramides.

The chemical composition of a Tasmanian collection of the marine bryozoan *Amathia wilsoni* Kirkpatrick (Vesiculariidae) has been investigated previously and a series of brominated alkaloids, amathamides A−G were isolated.[1−3] In a survey of the chemical composition of New Zealand marine bryozoans, we undertook an investigation of an extract of a New Zealand collection of *A. wilsoni*. We report here the isolation and structural elucidation of six novel alkaloids, amathaspiramides A−F (**1**−**6**), from this bryozoan. Amathaspiramide A (**1**) was acetylated to produce amathapiramide A monoacetate (**7**). Data from a single-crystal X-ray diffraction study of amathaspiramide F (**6**) and those from NOESY NMR experiments were used to infer the absolute configuration of the amathaspiramides. The biological activity of this series of alkaloids was investigated.



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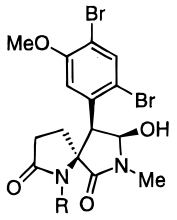
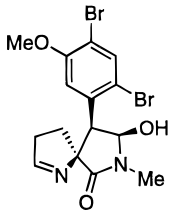
3: R^1 = H, R^2 = H
7: R^1 = Me, R^2 = Ac



2: R = Me
4: R = H



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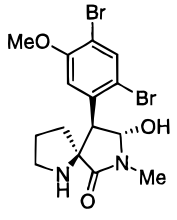

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Results and Discussion

A. wilsoni is a fairly common bryozoan of the coastal waters of New Zealand and southern Australia.[4] The bryozoan was collected by SCUBA from Barrett Reef in Wellington Harbor, off the North Island of New Zealand and identified as *A. wilsoni*. The frozen bryozoan was extracted with MeOH/CH_2Cl_2 (3:1). The extract gave a positive Mayer's test for alkaloids[5] and was subjected to repeated reversed-phase flash column chromatography to give amathaspiramide A (**1**) in 0.05% yield (based on the bryozoan wet weight). Further separation, using both gel permeation (LH-20) and reversed-phase HPLC, led to the isolation of amathaspiramides B−F (**2**−**6**) in 0.00075, 0.0021, 0.00025, 0.00038, and 0.0004% yield, respectively.

The structure of **1** was deduced by spectral analysis and confirmed by chemical modification. The electrospray mass spectrum of amathaspiramide A (**1**) in positive-ion mode at an exit cone voltage of +20 V contained the parent ion at 449 amu with an isotope pattern for the presence of two bromine atoms. At higher cone voltage (+80 V), further fragmentation was evident. HREIMS established the molecular formula $C_{16}H_{20}Br_2N_2O_3$.

Examination of the ^{1}H and ^{13}C NMR spectra of **1** indicated that it was related to the amathamides isolated previously but with some significant structural variations. The ^{1}H NMR spectrum of **1** (Table 1) exhibited 13 signals: two aromatic proton singlets, two mutually coupled one-proton doublets in the midfield region of the spectrum, a methoxy methyl signal, two *N*-methyl signals and six well-spread aliphatic proton multiplets. The ^{13}C NMR spectrum (Table 2) contained 16 signals, the multiplicities of which were established by a DEPT-135 NMR experiment and comprised one carbonyl resonance, six aromatic signals, and nine aliphatic carbon signals, including a quaternary carbon signal at 71.3 ppm.

Proton−proton and proton−carbon connectivities were established by COSY, gHSQC, and gHMBC NMR experiments. These experiments, and comparison with previously published NMR spectral data of amathamides,[1−3] established the presence of an aromatic ring with one methoxyl and two bromine substituents, an *N*-monosubstituted amide, and a 1-methyl pyrrolidine group. The chemical shifts of C-8 and C-9 and their attached protons suggested a single bond between them, and those of H-8 and C-8, at 5.12 and 82.4 ppm, respectively, were indicative of an attached oxygen at this position. The molecular formula required the presence of one more ring than in amathamides A−G.[1−3] That C-5 was a spiro carbon was inferred from gHMBC correlations to it from H-8 and H-9, which could only be observed if C-9 and C-5 were linked (Figure 1). One proton was left to be accounted for, implying a hydroxyl substituent at C-8.

Acetylation of **1** with acetic anhydride/pyridine to yield the monoacetate (**7**) confirmed this structure. The ^{1}H NMR

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Table 1. ^{1}H NMR Data (δ in ppm, J in Hz) for Amathaspiramides A−F (**1**−**6**) and Amathaspiramide A Monoacetate (**7**) in $CDCl_3$

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1			7.7 br s	6.8 br s			
2	2.73 α, m		2.62 α, m		7.7 br s	2.58 α, m	2.83 α, m
	2.90 *β*, m		3.08 *β*, m			3.03 *β*, m	2.90 *β*, m
3	1.32 α, m	1.85 α, m	1.62 α, m	2.03 α, m	2.45 α, m	1.83 α, m	1.32 α, m
	1.69 *β*, m	2.22 *β*, m	1.87 *β*, m	2.50 *β*, m	2.83 *β*, m	1.83 *β*, m	1.75 α, m
4	1.86 α, m	2.05 α, m	1.73 α, m	2.03 α, m	1.64 α, m	2.13 α, m	1.89 α, m
	2.31 *β*, m	2.56 *β*, m	2.17 *β*, m	2.50 *β*, m	2.39 *β*, m	2.13 *β*, m	2.40 *β*, m
8	5.15 d (5.5)	5.25 d (5.3)	5.07 d (4.8)	5.21 d (4.6)	5.11 d (4.4)	5.05 d (2.6)	6.28 d (5.7)
9	3.90 d (5.5)	4.17 d (5.3)	3.90 d (4.8)	4.04 d (4.6)	4.10 d (4.4)	3.72 d (2.6)	4.09 d (5.7)
12	7.70 s	7.78 s	7.77 s	7.82 s	7.75 s	7.74 s	7.72 s
15	7.67 s	6.83 s	7.75 s	7.25 s	7.27 s	6.72 s	7.64 s
16	3.84 s	3.82 s	3.87 s	3.81 s	3.82 s	3.81 s	3.87 s
17	2.94 s	3.07 s	3.01 s	3.03 s	3.06 s	3.00 s	2.96 s
18	2.48 s	2.92 s					2.57 s
OAc							1.94 s

Table 2. ^{13}C NMR Data (δ in ppm) for Amathaspiramides A−F (**1**−**6**) and Amathaspiramide A Monoacetate (**7**) in $CDCl_3$

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	57.4	176.2	48.1	173.7	170.4	47.7	57.6
3	23.6	29.6	26.6	27.9	38.0	26.3	23.8
4	37.0	30.5	34.0	29.6	25.0	39.0	37.6
5	71.3	70.7	70.2	65.8	85.0	70.3	70.7
6	175.2	171.7	176.7	178.7	173.9	176.9	175.3
8	82.6	82.1	84.2	83.7	85.2	88.0	82.6
9	51.3	52.5	51.5	50.9	51.6	57.9	49.9
10	134.6	132.5	134.3	132.1	133.6	137.7	133.7
11	117.1	116.8	117.6	117.2	117.3	116.0	117.4
12	135.0	136.3	136.1	136.3	136.0	136.3	135.4
13	111.0	112.5	112.0	112.7	111.9	111.5	111.5
14	154.1	155.2	155.0	155.2	154.7	155.5	154.1
15	118.3	115.3	115.2	115.6	116.0	111.9	117.5
16	56.2	56.8	56.5	56.6	56.4	56.5	56.2
17	27.7	28.2	27.7	28.2	28.0	27.6	28.5
18	36.5	28.0					36.5
OAc							169.7
							21.0

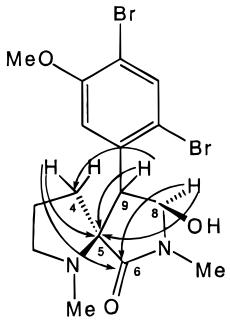

Figure 1. Selected gHMBC correlations for amathaspiramide A (**1**).

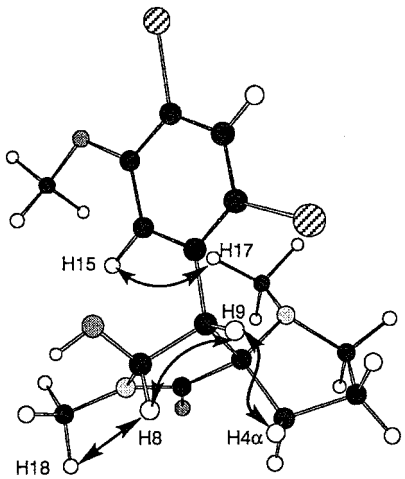

Figure 2. Minimum energy conformation of amathaspiramide A (**1**), calculated using Macromodel software,[6] showing selected NOESY correlations.

spectrum of **7** contained one extra acetate methyl peak at 1.94 ppm and the H-8 and H-9 signals were both shifted downfield by 1.13 and 0.19 ppm respectively (Table 1). The ^{13}C NMR spectrum of **7** (Table 2) contained two additional resonances at 21.0 and 169.7 ppm for the acetate functionality and a gHMBC correlation between H-8 and the acetate carbonyl resonance confirmed the site of acetylation (Table 3).

The relative configuration of **1** was established from coupling constant considerations and NOESY NMR experiments. The magnitude of the coupling constant between H-8 and H-9 (5.5 Hz) indicated that they were fixed in an eclipsed conformation by the ring. The lack of NOESY correlations between H-15 and H-8, H-9 and between H-15 and H-2, H-3, established the conformation of the aromatic ring. The configuration at the spiro carbon was also established from NOESY NMR experiments. Strong NOESY correlations observed between the H-17 methyl protons and H-15 could only be seen if the configuration at C-5 is as depicted. This was confirmed by the absence of any NOESY correlation between H-18 and H-9 and the observation of such a correlation between H-4α and H-9 (Figure 2).

The ^{1}H NMR spectrum of amathaspiramide B (**2**) (Table 1) resembled that of amathaspiramide A (**1**), but the two aromatic proton singlets were much further apart than those in the spectrum of **1** and there were only four aliphatic proton multiplets present. The ^{13}C NMR spectrum of **2** (Table 2) also resembled that of **1** in most respects but contained an additional carbonyl signal at 176.2 ppm and the chemical shifts of carbons on the pyrrolidine ring

Table 3. gHMBC NMR Data (H to C) for Amathaspiramides A−F (**1**−**6**) and Amathaspiramide A Monoacetate (**7**) in $CDCl_3$

proton	1	2	3	4	5	6	7
2			C-5, C-4		C-3, C-4, C-5	C-4, C-5	
3		C-2, C-5	C-4	C-5			
4	C-5, C-6	C-2, C-3, C-5, C-6, C-9	C-2, C-3, C-5, C-6, C-9		C-3, C-6	C-2, C-9	
8	C-5, C-6	C-5, C-6	C-5, C-6	C-5, C-6	C-5, C-6		C-5, C-6, C-19
9	C-4, C-5, C-8, C-10, C-11, C-15	C-4, C-5, C-8, C-10, C-11, C-15	C-4, C-5, C-8, C-10, C-11, C-15	C-5, C-8, C-10, C-11, C-15	C-4, C-5, C-8, C-10, C-11, C-15	C-8, C-10, C-11	C-4, C-5, C-8, C-10, C-11, C-15
12	C-10, C-11, C-13, C-14	C-10, C-11, C-13, C-14	C-10, C-11, C-13, C-14	C-10, C-11, C-13, C-14	C-10, C-11, C-13, C-14	C-10, C-11, C-13, C-14	C-11, C-13
15	C-9, C-13, C-14	C-9, C-13, C-14	C-9, C-13, C-14	C-9, C-13, C-14	C-9, C-10, C-13, C-14	C-9, C-13, C-14	C-13, C-14
16	C-14	C-14	C-14	C-14	C-14	C-14	C-14
17	C-6, C-8	C-6, C-8	C-6, C-8	C-6, C-8	C-6, C-8	C-6, C-8	C-6, C-8
18	C-2, C-5	C-2, C-5					C-2, C-5
20							C-19

differed somewhat. The electrospray mass spectrum of **2** showed the same isotope pattern as for **1** but a higher molecular mass. HREIMS established a molecular formula of $C_{16}H_{18}Br_2N_2O_4$, implying that a carbonyl replaced a methylene in the pyrrolidine ring. The position of the carbonyl was confirmed as C-2 by gHMBC correlations from H-18 to C-2 and from H-4 to C-5 and C-9 (Table 3).

The ^{1}H NMR spectrum of amathaspiramide C (**3**) (Table 1) contained one less *N*-methyl proton resonance than those of amathaspiramides A (**1**) and B (**2**) and the aliphatic multiplets integrated for six protons, as for **1**. In addition, a broad one-proton singlet at 7.7 ppm was present. The ^{13}C NMR spectrum of **3** (Table 2) also contained only one *N*-methyl carbon resonance. Electrospray mass spectrometry indicated a parent ion of 434 amu (14 amu less than **1**) and HREIMS confirmed a molecular formula of $C_{15}H_{18}Br_2N_2O_3$ (one carbon and two hydrogens less than for **1**). These data implied a structure which was as for **1** but without the *N*-methyl group in the pyrrolidine ring. gHMBC experiments confirmed this structure, with correlations from H-1 to C-4 and C-5 (Table 3).

The ^{1}H NMR spectrum of amathaspiramide D (**4**) (Table 1) was very similar to that of amathaspiramide B (**2**) except that the spectrum of **4** contained one less methyl signal than that of **2** and an additional broad singlet at 6.6 ppm was present. The ^{13}C NMR spectrum of **4** (Table 2) also contained only one *N*-methyl carbon resonance. Upfield shifts of 4.9 ppm for C-5 and 2.5 ppm for C-2 and a downfield shift of 7.0 ppm for C-6 over those of **2** were apparent. HREIMS indicated a molecular formula of $C_{15}H_{16}Br_2N_2O_4$. These results suggested that **4** was the *N*-1-demethyl derivative of **2**. This was again supported by the results of gHMBC experiments (Table 3).

The ^{1}H NMR spectrum of amathaspiramide E (**5**) (Table 1) contained a broad singlet at 7.7 ppm, a singlet at 7.27 ppm, and only two multiplets, at 2.43 and 2.83 ppm, but was otherwise similar to the spectrum of amathaspiramide C (**3**). The ^{13}C NMR spectrum of **5** (Table 2) was also very similar to that of **3** but contained one less signal in the aliphatic region of the spectrum which was replaced by a signal at 170.4 ppm. The signal for C-5 was shifted downfield by 15 ppm from 70.2 to 85.0 ppm. A DEPT-135 NMR experiment revealed that there were only two methylene carbons present, and that the carbon at 170.4 ppm was protonated. This was confirmed by a gHSQC correlation between the proton signal at 7.7 ppm and this carbon resonance. The same proton showed gHMBC correlations to C-3, C-4, and C-5, and there was also a correlation between H-9 and C-4 (Table 3). These results indicated that there was a double bond between N-1 and C-2, forming an imine, while the rest of the molecule was identical to amathaspiramide C (**3**). HRFABMS of **5** confirmed a molecular formula of $C_{15}H_{16}Br_2N_2O_3$.

The ^{1}H NMR spectrum of amathaspiramide F (**6**) (Table 1) was similar to that of amathaspiramide C (**3**), but the signal for H-15 was shifted 1 ppm upfield, while the signal for H-9 was shifted 0.2 ppm upfield. The coupling constant between H-8 and H-9 (2.6 Hz) was significantly less than that for all other amathaspiramides, suggesting a different dihedral angle between these protons. The ^{13}C NMR spectrum of **6** (Table 2) was also very similar to that of **3**, except for 4−6 ppm downfield shifts for C-4, C-8, and C-9 and a slight upfield shift for C-15. Both the gHSQC and gHMBC NMR experiment gave almost identical results to those of the same experiments run on **3** (Table 3). HRFABMS of **6** gave an $[MH]^+$ ion which confirmed that the molecular formula was $C_{15}H_{18}Br_2N_2O_3$, the same as that of **3**.

The structure of **6** was confirmed by a single-crystal X-ray diffraction study and was solved by direct methods. An ORTEP plot of the crystal structure of **6** (Figure 3) indicates that the molecule possesses 5*S*,8*S*,9*R* stereochemistry. Atomic coordinates and equivalent isotropic displacement parameters are given in Table 4. NOESY NMR experiments on **6**, especially a crucial correlation from H-15 to H-8, confirmed that the configuration at C-8 was the opposite of that in amathaspiramide C (**3**) (Figure 4). The NOESY NMR spectrum of **3** showed no correlation between H-15 and H-8, but a strong correlation between H-8 and H-9. This indicated that H-8 and H-9 were in a *Z* configuration for **3**, instead of in the *E* configuration as in **6**. A correlation between H-9 and H-4α in **3** indicated that C-5 and C-9 had the same relative stereochemistry as in **6** (Figure 4). Therefore, **3** possessed 5*S*,8*R*,9*R* stereochemistry and was thus epimeric at C-8 to **6**.

Amathaspiramide A (**1**) showed the same NOE enhancements as amathaspiramide C (**3**) when H-8 and H-9 were irradiated, indicating that **1** and **3** had the same relative configuration; therefore **1** also possessed 5*S*,8*R*,9*R* stereochemistry. Similarly, amathaspiramides B (**2**), D (**4**), and E (**5**) also gave the same NOESY correlations for H-8 and H-9 as **3**, indicating that they had the same stereochemistry.

Amathaspiramides A−C (**1**−**3**) and E (**5**) were assayed for P388 murine leukemic, antiviral/cytotoxic, and antimicrobial activity. Details of the assay procedures have been

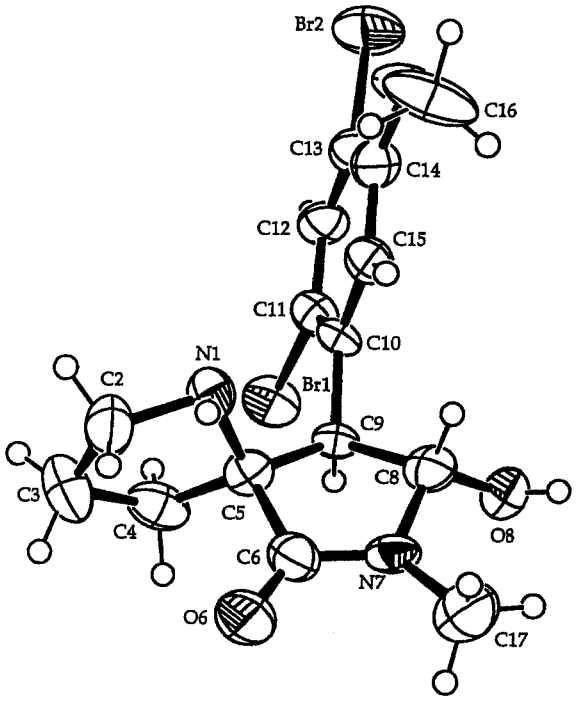

Figure 3. ORTEP diagram of amathaspiramide F (**6**).

Table 4. Atomic Coordinates [$\times 10^4$] and Equivalent Isotropic Displacement Parameters [Å^2 $\times$ 10^3] for Amathaspiramide F (**6**)

	x	*y*	*z*	*U*(eq)[a]
Br-1	1069 (1)	10003 (1)	2408 (2)	58 (1)
Br-2	112 (2)	13008 (1)	2130 (2)	84 (1)
N-1	−765 (7)	10058 (6)	−2460 (13)	45 (2)
N-7	−2200 (8)	9010 (6)	−446 (13)	41 (3)
O-6	−1541 (8)	8477 (5)	−2566 (13)	66 (3)
O-8	−2289 (7)	9499 (5)	2098 (11)	49 (2)
O-14	−1511 (9)	12479 (5)	90 (14)	67 (3)
C-2	−191 (13)	9759 (9)	−3725 (18)	66 (5)
C-3	606 (13)	9244 (10)	−3175 (20)	71 (5)
C-4	360 (11)	9154 (8)	−1470 (18)	54 (4)
C-5	−672 (10)	9489 (7)	−1228 (16)	40 (3)
C-6	−1503 (12)	8922 (8)	−1485 (16)	49 (4)
C-8	−2036 (11)	9650 (7)	576 (15)	45 (4)
C-9	−924 (9)	9778 (6)	350 (13)	27 (3)
C-10	−627 (10)	10570 (7)	767 (14)	34 (3)
C-11	195 (10)	10740 (7)	1650 (14)	41 (3)
C-12	410 (10)	11460 (7)	2054 (16)	46 (4)
C-13	−188 (11)	12027 (7)	1494 (15)	46 (4)
C-14	−960 (10)	11902 (7)	569 (15)	41 (3)
C-15	−1178 (11)	11165 (7)	218 (16)	46 (4)
C-16	−2326 (15)	12341 (9)	−942 (22)	89 (7)
C-17	−3182 (13)	8673 (9)	−517 (19)	73 (5)
C-18	3367 (11)	8201 (8)	2106 (21)	71 (5)
Cl-1	4604 (4)	8133 (3)	1535 (9)	126 (2)
Cl-2	3231 (5)	8893 (3)	3496 (6)	100 (2)
Cl-3	2626 (5)	8414 (4)	518 (7)	113 (2)

[a] (*U*(eq) is defined as one-third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor).

reported elsewhere.[7,8] None of the amathaspiramides showed any significant activity in the P388 assay (IC_{50} values > 12.5 μg/mL). Amathaspiramide E (**5**) exhibited strong activity (4+, 40 μg/well) in the antiviral assay against *Polio* virus Type 1 (Pfizer vaccine strain) in addition to moderate cytotoxicity (1+, 40 μg/well) to the BSC-1 cells used in this assay, while amathaspiramide A (**1**) displayed moderate cytotoxicity (1+, 40 μg/well) to these cells also.[7,8] Amathaspiramides B (**2**) and C (**3**) were inactive at the concentrations tested. In the antimicrobial assay, amathaspiramides A (**1**) and E (**5**) displayed mild activity (1 mm inhibition zone, 60 μg/disk) against the Gram-positive bacterium *Bacillus subtilis* and the fungus *Trichophyton mentagrophytes*, while amathaspiramides B (**2**) and C (**3**) were again inactive at the concentrations tested.[7,8]

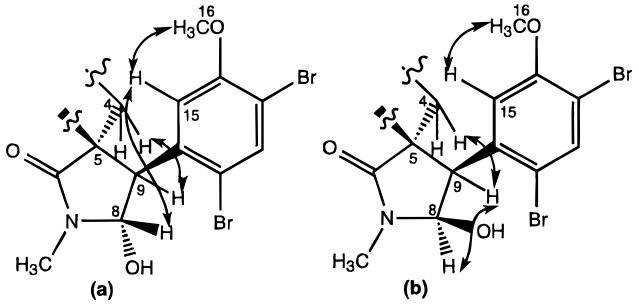

Figure 4. Selected NOESY correlations for substructures of amathaspiramides F (**6**) (**a**) and C (**3**) (**b**).

A thorough investigation of the bryozoan extract showed that no amathamides were present in the extract. Previous studies of Tasmanian collections of *A. wilsoni* found that there is little variation in alkaloid content between local populations but there is some variability between populations from different locations.[3,9] That the New Zealand collection of *A. wilsoni* contains only amathaspiramides is possibly a reflection of genetic variability or different environmental conditions for the New Zealand populations of the bryozoan. Investigations of other samples of *A. wilsoni* collected from the same location but at different times, showed identical alkaloid content, and investigations of collections made at different sites around New Zealand are now in progress. A direct comparison of samples of *A. wilsoni* from Tasmania and New Zealand indicated that by standard morphological criteria, both forms are the same species. The Tasmanian specimen is somewhat more robust, but this is the sort of variation expected from differences in nutrient and local hydrographic conditions (D. P. Gordon, personal communication, 1997).

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were determined on a 7.1 T instrument operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts are referenced in $CDCl_3$ to 7.26 ppm (residual $CHCl_3$) and 77.0 ppm, respectively. Homonuclear connectivities were determined with the COSY experiment, and heteronuclear ^{1}H−^{13}C connectivities were determined by gHSQC and gHMBC experiments on a 9.4 T instrument operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. NOESY spectra were also determined on this instrument. Electrospray mass spectra were measured on a VG Platform II machine operating in positive ion mode with the cone voltage set at 20 V. Higher cone voltages (80−100 V) gave a characteristic fragmentation pattern for the amathaspiramides. A VG 7070E mass spectrometer was used to measure HRMS with either electron-impact ionization or fast-atom bombardment (Ar). Single-crystal X-ray diffraction data was collected on a Siemens P4 diffractometer with Mo Kα radiation. UV spectra were measured on an Hitachi 150−20 spectrophotometer and IR spectra were measured on a Perkin-Elmer 1610 FTIR instrument. Optical rotations were measured for methanol solutions on a JASCO J- 20C automatic recording spectrophotometer. C_{18} HPLC was carried out using a Perkin-Elmer 410 pump, an Alltech Econosil column with 30% aqueous methanol as solvent, and a linear 206PHD UV detector. Column chromatography was performed using silica gel (Davisil 35−70 μm) and C_{18} reversed-phase material prepared by coating silica gel with *n*-octadecyltrichlorosilane after the method of Evans et al.[10] Gel permeation was carried out using Sephadex LH-20 (Pharmacia Fine Chemicals) packed in methanol.

Animal Material. Colonies of *A. wilsoni* (2684 g in total, wet weight) were collected by Scuba in February 1993 and February 1994 from Barrett Reef, Wellington, New Zealand and stored frozen. Voucher specimens 93BR01-06 and 94BR01-04 are held at the Department of Chemistry, University of Waikato. The bryozoan was identified by Dr. D. P. Gordon.

Extraction and Isolation. In a typical extraction, subsamples of the bryozoan were extracted three times in MeOH/CH_2Cl_2 (1:3) (500 mL total per 100 g of wet bryozoan) with a hand-held blender. The resulting extracts were combined and filtered under vacuum, then the solvent removed in vacuo followed by lyophilization.

A. wilsoni (314 g wet weight) was extracted by the method described above. The crude extract (13.4 g) was fractionated by reversed-phase flash column chromatography on C_{18} silica gel (60 g) using a steep step-gradient from H_2O to MeOH to CH_2Cl_2. Several fractions from this column contained pure amathaspiramide A (**1**) (153.3 mg) by NMR spectroscopy. Other fractions (70 mg) containing amathaspiramides A (**1**), C (**3**), and F (**6**) by TLC [silica gel, EtOAc−acetone (5:1), UV light 254 nm] were combined and separated by gel permeation on Sephadex LH-20 (40 g) using MeOH as solvent. Fractions from this column containing amathaspiramides by TLC were combined (52 mg) and submitted to HPLC [Alltech Econosil C_{18}, MeOH−H_2O (9:1) + 0.1% TFA, flow rate 5 mL/min, UV detection at 220 nm]. Fraction 1 from the HPLC separation (6.5 mg) was subjected to gel permeation on Sephadex LH-20 in MeOH (35 g) to remove TFA to yield amathaspiramide C (**3**) (6.3 mg) while fractions 2 (2.5 mg) and 3 (17.3 mg) were subjected to gel permeation in a manner similar to yield amathaspiramide F (**6**) (1.4 mg) and amathaspiramide A (**1**) (9.8 mg), respectively. The latter was combined with the amathaspiramide A already isolated to yield a total of 163.1 mg of **1**.

Fractions from the initial reversed-phase flash column containing amathaspiramides B (**2**) and D (**4**) by TLC (73 mg) were combined and subjected to gel permeation followed by HPLC [Alltech Econosil C_{18}, MeOH−H_2O (3:2), flow rate 5 mL/min, UV detection at 220 nm] to yield amathaspiramide B (**2**) (3.0 mg) and amathaspiramide D (**4**) (1.0 mg).

A fraction from the initial reversed-phase flash column was subjected to further reversed-phase flash column chromatography, gel permeation and HPLC in a similar manner to yield amathaspiramide E (**5**) (1.5 mg).

Amathaspiramide A (1). Amorphous, white solid, pure by TLC analysis [silica gel, EtOAc−acetone (5:1), UV light 254 nm, $R_f = 0.86$], $[\alpha]^{25}_D$ −3° (*c* 0.0045, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.20), 289 (3.34), 295 (3.36) nm; IR (smear) ν_{max} 3400 br, 1672, 1467, 1442, 1249, 1203, 1140, 1052 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m/z* (+80 V) 451, 449, 447 (31, 64, 35) $[MH]^+$, 433, 431, 429 (11, 23, 11), 405, 403, 401 (50, 100, 50), 364, 362, 360 (31, 65, 34), 324, 322 (31, 31); EIMS *m/z* 450, 448, 446 $[M]^+$, 432, 430, 428 $[M - H_2O]^+$, 369, 367 $[M - Br]^+$, 323, 321, 311, 308, 141, 57; HREIMS *m/z* 449.9793 (calcd for $C_{16}H_{20}{}^{81}Br_2N_2O_3$, 449.9800), 447.9812 (calcd for $C_{16}H_{20}{}^{79}Br^{81}BrN_2O_3$, 447.9820), 445.9837 (calcd for $C_{16}H_{20}{}^{79}Br_2N_2O_3$, 445.9841).

Amathaspiramide B (2). Amorphous, colorless solid, pure by TLC analysis [silica gel, EtOAc-acetone (5:1), UV light 254 nm, $R_f = 0.40$], $[\alpha]^{25}_D$ −13° (*c* 0.0022, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.50), 310 (4.38), 436 (4.60) nm; IR (smear) ν_{max} 3389 br, 1680, 1470, 1391, 1370, 1250, 1098, 1053 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m/z* (+80 V) 487, 485, 483 (19, 37, 20), $[M + Na]^+$, 465, 463, 460 (50, 100, 51), $[MH]^+$, 447, 445, 443 (17, 32, 17), 419, 417, 415 (46, 93, 47), 378, 376, 374 (26, 47, 33); HREIMS *m/z* 463.9614 (calcd for $C_{16}H_{18}{}^{81}Br_2N_2O_4$, 463.9592), 461.9615 (calcd for $C_{16}H_{18}{}^{79}Br^{81}BrN_2O_4$, 461.9613), 459.9599 (calcd for $C_{16}H_{18}{}^{79}Br_2N_2O_4$, 459.9633).

Amathaspiramide C (3). Amorphous, colorless solid, pure by TLC analysis [silica gel, EtOAc-acetone (5:1), UV light 254 nm, $R_f = 0.49$], $[\alpha]^{25}_D$ −2° (*c* 0.0046, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.16), 289 (3.15), 294 (3.15) nm; IR (smear) ν_{max} 3422 br, 1682, 1474, 1441, 1208, 1139, 1054 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m/z* (+80 V) 459, 457, 455 (13, 24, 13) $[M + Na]^+$, 437, 435, 433 (16, 31, 17) $[MH]^+$, 419, 417, 415 (5, 9, 5), 391, 389, 387 (49, 100, 50), 350, 348, 346 (33, 68, 35), 269, 267 (14, 14); HRFABMS *m/z* 436.9725 $[MH]^+$ (calcd for $C_{15}H_{19}{}^{81}Br_2N_2O_3$, 436.9721), 434.9738 (calcd for $C_{15}H_{19}{}^{79}Br^{81}BrN_2O_3$, 434.9742), 432.9732 (calcd for $C_{15}H_{19}{}^{79}Br_2N_2O_3$, 432.9762).

Amathaspiramide D (4). Amorphous, white solid, pure by TLC analysis [silica gel, EtOAc−acetone (5:1), UV light 254 nm, $R_f = 0.24$], $[\alpha]^{25}_D$ −44° (*c* 0.0022, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.03), 289 (3.22), 295 (3.22) nm; IR (smear) ν_{max} 3411 br, 1691, 1477, 1369, 1253, 1050 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m/z* (+80 V) 473, 471, 469 (52, 100, 51) $[M + Na]^+$, 433, 431, 429 (7, 14, 6) $[MH - H_2O]^+$; HREIMS *m/z* 449.9449 (calcd for $C_{15}H_{16}{}^{81}Br_2N_2O_4$, 449.9436), 447.9509 (calcd for $C_{15}H_{16}{}^{79}Br^{81}BrN_2O_4$, 447.9456), 445.9516 (calcd for $C_{15}H_{16}{}^{79}Br_2N_2O_4$, 445.9477).

Amathaspiramide E (5). Amorphous, colorless solid, pure by TLC analysis [silica gel, EtOAc-acetone (5:1), UV light 254 nm, $R_f = 0.27$], $[\alpha]^{25}_D$ −21° (*c* 0.0023, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.13), 289 (3.17), 295 (3.17) nm; IR (smear) ν_{max} 3367 br, 1692, 1468, 1447, 1367, 1250, 1053 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m/z* (+20 V) 467, 465, 463 (51, 100, 50) $[M + Na]^+$, 451, 449, 447 (25, 52, 28) $[MH + CH_4]^+$, 437, 435, 433, 431 (25, 55, 40, 10) overlapping $[MH]^+$ and $[MH + H_2]^+$, (+80 V) 451, 449, 447, 445 (15, 47, 54, 22), 437, 435, 433, 431 (16, 37, 35, 20), 417, 415, 413(57, 100, 54) $[MH - H_2O]^+$, 405, 403, 401 (24, 48, 25) $[(MH + CH_4) - H_2O - CO]^+$, broad peak centered on 388 (48), 364, 362, 360 (11, 25, 17), 350, 348, 346 (12, 25, 12); HRFABMS *m/z* 434.9601 $[MH]^+$ (calcd for $C_{15}H_{17}{}^{81}Br_2N_2O_3$, 434.9565), 432.9617 (calcd for $C_{15}H_{17}{}^{79}Br^{81}BrN_2O_3$, 432.9585), 430.9549 (calcd for $C_{15}H_{17}{}^{79}Br_2N_2O_3$, 430.9606), 414.9470 (calcd for $C_{15}H_{15}{}^{79}Br^{81}BrN_2O_2$, 414.9480) $[MH-H_2O]^+$.

Amathaspiramide F (6). Amorphous, colorless solid, pure by TLC analysis [silica gel, EtOAc−acetone (5:1), UV light 254 nm, $R_f = 0.20$], [α] 0°, [α] −10° (*c* 0.0023, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.12), 290 (3.08), 296 (3.08) nm; IR (smear) ν_{max} 3422 br, 1681, 1474, 1441, 1207, 1140, 1054 cm^{-1}; for ^1H and ^{13}C NMR see Tables 1 and 2; ESMS *m/z* (+80 V) 437, 435, 433 (52, 100, 52) $[MH]^+$, 391, 389, 387 (6, 12, 6), 378, 376, 374 (8, 16, 8), 350, 348, 346 (12, 24, 13), 369, 367 (9, 9); HRFABMS *m/z* 436.9742 $[MH]^+$ (calcd for $C_{15}H_{19}{}^{81}Br_2N_2O_3$, 436.9721), 434.9734 (calcd for $C_{15}H_{19}{}^{79}Br^{81}BrN_2O_3$, 434.9742), 432.9715 (calcd for $C_{15}H_{19}{}^{79}Br_2N_2O_3$, 432.9762).

Preparation of Amathaspiramide A Monoacetate (7). Amathaspiramide A (**1**) (5.3 mg) was dissolved in redistilled pyridine (0.5 mL) to which redistilled acetic anhydride (0.5 mL) was added. After overnight stirring, the solvent was removed as an azeotrope with toluene under reduced pressure to yield amathaspiramide A monoacetate (**7**) (3.5 mg) as an amorphous solid. UV (MeOH) λ_{max} (log ϵ) 210 (4.24), 290 (3.43), 296 (3.43) nm; IR ν_{max} (smear) 3400 br, 1712, 1472, 1367, 1250, 1053, 1019 cm^{-1}; for ^1H and ^{13}C NMR see Tables 1 and 2; ESMS *m/z* (+80 V) 493, 491, 489 (23, 47, 28), 465, 463, 461 (24, 48, 27), 433, 431, 429 (29, 57, 32), 405, 403, 401 (53, 100, 51), 364, 362, 360 (32, 63, 33), 324, 322 (29, 29); HRCIMS *m/z* 492.9955 (calcd for $C_{18}H_{22}{}^{81}Br_2N_2O_4$, 492.9984), 490.9994 (calcd for $C_{18}H_{22}{}^{79}Br^{81}BrN_2O_4$, 491.0004), 489.0011 (calcd for $C_{18}H_{22}{}^{79}Br_2N_2O_4$, 489.0025).

X-ray Crystallography of Amathaspiramide F (6). Thin needles of **6** were obtained from $CDCl_3$ by slow evaporation. Preliminary precession photography indicated that the crystals of **6** were orthorhombic. The unit cell dimensions and intensity data were obtained on a Siemens SMART diffractometer. The data collection nominally covered over a hemisphere of reciprocal space, by a combination of three sets of exposures. Each set had a different ϕ angle for the crystal and each exposure covered 0.3° in ω. The crystal to detector distance was 5.0 cm. The data sets were corrected empirically for absorption using SADABS.[11]

The structure was solved by direct methods using the SHELXS 86 structure- solving package[12] and refined with the SHELXL-93 package[13] using full matrix least-squares based on F^2. All non-hydrogen atoms were treated anisotropically and hydrogen atoms were included in their calculated positions.[14]

Crystal data: $C_{15}H_{18}Br_2N_2O_3.CDCl_3$, M_r = 553.49. Orthorhombic, space group $P2_12_12_1$ with a = 13.4540(1) Å, b = 18.1313(5) Å, c = 8.7092(1) Å, V = 2124.51(7) Å^3, Z = 4, D_c = 1.727 g cm^{-3}, F(000) 1092, μ(Mo Kα) = 4.211 mm^{-1}. A total of 10 536 reflections were collected at 203 K in the range 1.88 < θ < 23.99°, corresponding to 3322 unique data (R_{int} = 0.0863), $T_{max,min}$ 0.2869, 0.2217. The refinement converged with R1 = 0.0850 (for 2431 data with $I > 2\sigma(I)$) and R1 = 0.1311, wR2 = 0.1816, GoF 0.994 (all data). A Flack χ parameter of 0.00(3) indicated that the correct polarity of the space group had been selected and that the observed relative configuration is the absolute configuration of **6**. The largest features in a final difference map were +0.478/−0.407 e Å^{-3}.

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(14) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax: +44-(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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