Polymastiamides B−F, Novel Steroid/Amino Acid Conjugates Isolated from the Norwegian Marine Sponge *Polymastia boletiformis*

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Received September 1, 1995[⊗]

The structures of polymastiamides B−F (**2**−**6**), five new steroid/amino acid conjugates isolated from the Norwegian marine sponge *Polymastia boletiformis*, have been solved by spectroscopic analysis of their desulfated methyl esters **8**−**12**, respectively. Polymastiamides were found to undergo double-bond migration during treatment with MeOH/HCl to give the $\Delta^{14,15}$ artifacts **13** to **16**. A C-ring benzenoid artifact **17** was also obtained from the methylation reaction.

Marine sponges are a rich source of steroids with highly functionalized nuclei and modified side chains.[1−4] We recently reported the isolation of polymastiamide A (**1**) from the Norwegian sponge *Polymastia boletiformis* (Lamarck, 1815; order: Hardromerida; family: Polymastiidae).[5] Polymastiamide A represented the first example of a naturally occurring steroid with a side chain containing an amide linkage between a carboxyl group on the steroid and the amino functionality of an α-amino acid. Further investigations of the extracts of *P. boletiformis* have resulted in the isolation of a number of minor analogues of polymastiamide A, whose structures are reported below.

Results and Discussion

Specimens of *Polymastia boletiformis* were collected by hand using SCUBA off Korsnes Peninsula, Norway, in July 1992. The MeOH extract of the sponge was partitioned between a H_2O/MeOH mixture and hexanes, and the H_2O/MeOH-soluble materials were chromatographed on Sephadex LH-20 to give several fractions (see Experimental Section). ^{1}H-NMR analysis indicated that the major fraction contained a complex mixture of steroidal compounds containing aromatic substructures. The mixture was further fractionated by reversed-phase silica column and repeated Sephadex LH-20 column chromatographies to give pure polymastiamide A (**1**) and complex mixtures of the other closely related metabolites.

Once the structure of polymastiamide A (**1**) was in hand, it was apparent that the difficulty in separating the related polymastiamides could be attributed to the presence of the sulfate and carboxylic acid functionalities, which made these molecules extremely polar. In order to exploit the convenience and efficiency of normal-phase HPLC purification, fractions containing inseparable mixtures of polymastiamides A to F (**1**−**6**) were combined, methylated, desulfated using MeOH/HCl, and separated by normal-phase HPLC to give pure samples of the desulfated methyl ester derivatives **7**−**12**, respectively. Thus, polymastiamides B to F (**2**−**6**) were obtained only as their methyl ester derivatives **8**−**12**, which were subjected to spectroscopic analysis. The methylation reaction and HPLC fractionation also yielded the rearranged products **13** to **16** and a C-ring benzenoid sterol **17** (Chart 1).

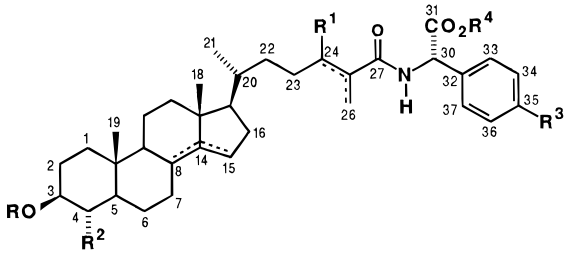
Polymastiamide B methyl ester (**8**) was isolated as a colorless glass that gave a parent ion in the HREIMS at *m*/*z* 605.40806, appropriate for a molecular formula of $C_{38}H_{55}NO_5$. The molecular formula of **8** contained one carbon and two hydrogen atoms less than the molecular formula of polymastiamide A methyl ester (**7**). The ^{1}H-NMR spectrum of methyl ester **8** displayed a close resemblance to that of compound **7**, particularly in the downfield region. For example, a pair of doublets at δ 6.85 (H34/36) and 7.27 (H33/H37) assigned to the aromatic ring, the NH proton doublet at δ 6.65, a methine proton resonance at δ 5.55 (H30), and two methoxy singlets at δ 3.77 (Me38) and 3.72 (Me39) in the ^{1}H-NMR spectrum of **8** had nearly identical chemical shifts and coupling constants as the corresponding resonances in the ^{1}H-NMR spectrum of **7**. These similarities in the ^{1}H NMR spectra of **7** and **8** implied that compound **8** also contained a (*p*-methoxyphenyl)-glycine methyl ester fragment. The most significant difference in the ^{1}H-NMR spectra of **7** and **8** was absence in the spectrum of **8** of the methyl doublet at δ 0.97 assigned to the Me29 protons in the ^{1}H-NMR spectrum of **7**. Another noticeable difference was the downfield shift of the carbinol proton resonance assigned to H3 from δ 3.11 in **7** to δ 3.60 in **8.** This evidence suggested that compound **8** differed from **7** simply by the absence of the Me29 methyl group at the C4 position. The upfield shift of the C3 resonance from δ 76.5 in **7** to δ 71.3 in **8**, and the presence of only four upfield methyl carbon resonances in the ^{13}C-NMR and APT spectra of methyl ester **8** were in good agreement with the lack of a methyl substituent at C4. Detailed analysis of the COSY, HMQC, HMBC, and NOE data acquired for **8**, and a comparison of the ^{13}C-NMR assignments for **7** and **8** (Table 1) confirmed the proposed structure and relative stereochemistry for **8**.

Polymastiamide C methyl ester (**9**) was obtained as a colorless solid that gave a parent ion in the HREIMS at *m*/*z* 605.40820, appropriate for a molecular formula of $C_{38}H_{55}NO_5$, indicating that it also contained one carbon and two hydrogen atoms less than polymastiamide A methyl ester (**7**). The resonances assigned to the steroidal nucleus and the *p*-methoxyphenylglycine methyl ester fragment in the ^{1}H-and ^{13}C-NMR spectra of **9** showed a close correspondence with the resonances assigned to the same fragments in **7** (Tables 1 and 2), indicating that the steroidal nucleus and *p*-methoxyphenylglycine methyl ester fragments were identical in **7** and **9**. Detailed analysis of COSY, HMQC, HMBC, and APT data obtained for **9** established the side chain substructure. The COSY spectrum showed a correlation

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1996.

0163-3864/96/3259-0379$12.00/0

Chart 1



Compound	R	R^1	R^2	R^3	R^4	Δ
1	SO_3^-	Me	Me	OMe	H	$\Delta^{8,14}$, $\Delta^{25,26}$
2	SO_3^-	Me	H	OMe	H	$\Delta^{8,14}$, $\Delta^{25,26}$
3	SO_3^-	H	Me	OMe	H	$\Delta^{8,14}$, $\Delta^{24,25}$
4	SO_3^-	H	H	OMe	H	$\Delta^{8,14}$, $\Delta^{24,25}$
5	SO_3^-	Me	Me	H	H	$\Delta^{8,14}$, $\Delta^{25,26}$
6	SO_3^-	H	Me	H	H	$\Delta^{8,14}$, $\Delta^{24,25}$
7	H	Me	Me	OMe	Me	$\Delta^{8,14}$, $\Delta^{25,26}$
8	H	Me	H	OMe	Me	$\Delta^{8,14}$, $\Delta^{25,26}$
9	H	H	Me	OMe	Me	$\Delta^{8,14}$, $\Delta^{24,25}$
10	H	H	H	OMe	Me	$\Delta^{8,14}$, $\Delta^{24,25}$
11	H	Me	Me	H	Me	$\Delta^{8,14}$, $\Delta^{25,26}$
12	H	H	Me	H	Me	$\Delta^{8,14}$, $\Delta^{24,25}$
13	H	Me	Me	OMe	Me	$\Delta^{15,14}$, $\Delta^{25,26}$
14	H	Me	H	OMe	Me	$\Delta^{15,14}$, $\Delta^{25,26}$
15	H	H	Me	OMe	Me	$\Delta^{15,14}$, $\Delta^{24,25}$
16	H	H	Me	H	Me	$\Delta^{15,14}$, $\Delta^{24,25}$

between a broad methyl singlet (δ 1.83, Me26) and a broad triplet at δ 6.38 (H24) that was correlated in the HMQC spectrum to an olefinic methine carbon at δ 137.9 (C24). The olefinic methine proton at δ 6.38 (H24) showed additional COSY correlations to a pair of allylic methylene protons at δ 2.17 and 2.01 (H23/H23′) that showed further COSY correlations to another pair of methylene protons at δ 1.51 and 1.20 (H22/H22′). HMQC correlations were observed between the methylene protons at δ 1.51 and 1.20 (H22/H22′) and a carbon at δ 34.6 (C22). The methylene carbon resonance at δ 34.6 (C22) was correlated to a methyl proton doublet at δ 0.94 (Me21, $J = 6.6$) in the HMBC spectrum of **9**. Further HMBC correlations observed between the allylic methyl resonance at δ 1.83 (Me26) and the olefinic methine carbon at δ 137.9 (C24), a fully substituted olefinic carbon at δ 129.8 (C25), and a carbonyl carbon at δ 168.5 (C27) completed the NMR assignments for the side chain substructure and revealed the existence of the α-methyl-α,β-unsaturated carbonyl system. An HMBC correlation between the NH proton resonance at δ 6.59 and the carbonyl resonance at δ 168.5 (C27) confirmed that the (*p*-methoxyphenyl)-glycine fragment was linked to the α,β-unsaturated carbonyl via an amide bond. The chemical shift of the ^{13}C-NMR resonance (δ 12.5) assigned to the olefinic methyl Me26,[6] and a lack of NOE or ROESY correlations between Me26 and the olefinic proton H24 (δ 6.38) indicated that the alkene in the side chain at $\Delta^{24,25}$ had *E*-configuration.

Polymastiamide D methyl ester (**10**) was obtained as a colorless solid that gave a parent ion in the HREIMS at *m/z* 591.39203, corresponding to a molecular formula of $C_{37}H_{53}NO_5$, which had one carbon and two hydrogen atoms less than the molecular formula of polymastiamide C methyl ester (**9**). The ^{1}H-NMR spectrum of compound **10** was nearly identical to that of **9**, except that the spectrum of **10** lacked the Me29 resonance at δ 0.96 present in the spectrum of **9** and the carbinol methine resonance (H3) in the spectrum of **10** was shifted downfield (δ 3.60) relative to the corresponding resonance in the spectrum of **9** (δ 3.10) (Table 2) . It was evident from the observed differences in the ^{1}H-NMR spectra of **9** and **10**, that methyl ester **10** differed from **9** simply by the absence of the Me29 methyl group at the C4 position. Comparison of the ^{13}C-NMR data obtained for **10** with the ^{13}C-NMR data obtained for **9** confirmed the absence of Me29 in **10** (Table 1). Detailed analysis of the COSY, HMQC, HMBC, and ROESY data for **10** led to complete proton, carbon, and relative stereochemical assignments (Tables 1 and 2).

Polymastiamide E methyl ester (**11**) was obtained as a colorless solid. The HREIMS spectrum of compound **11** gave a parent ion at *m/z* 589.41223, appropriate for a molecular formula of $C_{38}H_{55}NO_4$, differing from that of methyl ester **7** only by loss of a CH_2O unit. Most features of the ^{1}H NMR spectrum of methyl ester **11** were essentially identical with those of the ^{1}H-NMR spectrum of methyl ester **7** (Table 2). The only notable difference in the spectra of the two compounds was that a pair of doublets at δ 7.28 (H33/H37) and 6.86 (H34/36) and a sharp methoxy singlet at δ 3.77 (Me38), assigned to the *p*-methoxyphenyl group, in the spectrum of **7** were replaced by a cluster of aromatic protons integrating for 5 protons at δ 7.35 in the spectrum of **11**. This evidence suggests that methyl ester **11** simply contains a phenylglycine methyl ester fragment. The COSY data collected for **11** are consistent with the proposed structure.

Polymastiamide F methyl ester (**12**) was isolated as a colorless solid that gave a parent ion in the HREIMS at *m/z* 575.39667, appropriate for a molecular formula of $C_{37}H_{53}NO_4$, differing from that of polymastiamide C methyl ester (**9**) simply by loss of a CH_2O unit. The

Table 1. ^{13}C NMR Spectral Data (δ) in $CDCl_3$ at 500 MHz

	compound									
C no.	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
1	36.3	36.5	36.3	36.5	36.3	36.8	37.1	36.8	36.8	36.5
2	31.2	31.6	31.2	31.6	31.2	31.0	31.5	31.0	30.9	31.4
3	76.5	71.3	76.5	71.3	76.5	76.5	71.3	76.5	76.5	76.4
4	39.7	38.3	39.7	38.3	39.7	39.1	38.4	39.1	39.1	39.5
5	50.7	44.3	50.6	44.3	50.7	50.5	44.4	50.5	50.5	46.5
6	24.9	28.9	24.8	28.9	24.9	23.9	28.5	23.9	23.9	21.0
7	29.7	29.6	29.6	29.6	29.7	30.2	30.1	30.1	30.1	31.4
8	126.0	126.3	126.2	126.6	126.2	34.4	35.1	34.4	34.4	128.5
9	49.4	49.3	49.4	49.3	49.4	53.8	53.7	53.8	53.8	145.7
10	37.5	36.8	37.5	36.8	37.5	36.2	35.7	36.2	36.2	36.5
11	20.0	19.9	19.9	20.0	19.9	21.7	21.9	21.7	21.7	124.5
12	37.4	37.2	37.3	37.3	37.3	42.4	42.4	42.4	42.4	130.4
13	42.6	42.7	42.7	42.8	42.7	47.0	47.3	47.1	47.1	141.9
14	142.1	142.7	141.9	142.4	141.9	155.5	155.4	155.6	155.6	142.8
15	25.7	25.8	25.7	25.8	25.7	116.9	117.0	116.8	116.8	27.0
16	27.0	27.0	27.0	27.0	27.0	35.5	35.5	35.5	35.5	24.5
17	56.6	56.6	56.6	56.7	56.6	58.4	58.6	58.5	58.5	48.2
18	18.1	18.2	18.2	18.3	18.2	16.8	16.8	16.8	16.8	19.2
19	13.9	12.8	13.9	12.8	13.9	13.1	12.0	13.1	13.1	23.0
20	34.5	34.5	34.3	34.3	34.3	33.9	34.0	33.8	33.8	36.0
21	19.1	19.1	18.9	18.9	18.9	18.9	19.0	18.8	18.8	14.3
22	33.1	33.1	34.6	34.7	34.7	33.1	33.3	34.8	34.8	33.7
23	31.8	31.78	25.1	25.1	25.1	31.9	32.1	25.1	25.1	33.8
24	35.8	35.8	137.9	137.8	137.9	35.7	35.9	137.8	137.9	35.8
25	150.9	150.9	129.8	129.8	129.8	150.9	151.1	129.8	129.8	151.0
26	115.6	115.8	12.5	12.5	12.5	115.6	115.6	12.5	12.5	115.6
27	168.9	169.0	168.5	168.5	168.5	168.9	168.9	168.5	168.5	168.9
28	19.8	19.7				19.8	19.8			19.4
29	15.2		15.2		15.2	15.2		15.2	15.2	15.2
30	55.8	55.8	56.0	56.0	56.6	55.8	55.9	56.0	56.6	56.0
31	171.7	171.7	171.9	171.9	171.7	171.7	171.7	171.9	171.7	171.7
32	128.7	128.7	128.8	128.9	136.8	128.7	128.9	128.8	136.7	128.7
33	128.5	128.5	128.6	128.6	127.3	128.4	128.5	128.6	127.3	128.5
34	114.4	114.4	114.4	114.4	129.2	114.4	114.5	114.4	129.0	114.4
35	159.8	159.8	159.7	159.7	128.5	159.7	159.8	159.7	128.5	159.8
36	114.4	114.4	114.4	114.4	129.2	114.4	114.5	114.4	129.0	114.4
37	128.5	128.5	128.6	128.6	127.3	128.4	128.5	128.6	127.3	128.5
38	55.3	55.3	55.3	55.3		55.3	55.3	55.3		55.3
39	52.7	52.7	52.7	52.7	52.8	52.7	52.6	52.7	52.8	52.7

^1H-NMR spectrum of methyl ester **12** was nearly identical with that of **9** except that a five-proton cluster of resonances at ca. δ 7.35 in the spectrum of **12** replaced a pair of doublets at δ 7.28 (H33/H37) and 6.86 (H34/36) and a sharp methoxy singlet at δ 3.77 (Me38) in the spectrum of **9**. It was apparent from the ^1H-NMR data that methyl ester **12** was simply the phenylglycine analogue of methyl ester **9**. The ^{13}C, COSY, HMQC, HMBC, and APT data obtained for methyl ester **12** were in complete agreement with the proposed structure (Tables 1 and 2).

The *p*-methoxyphenylglycine and phenylglycine residues in polymastiamides B to F (**2**–**6**) were assumed to have the L configuration found for the (*p*-methoxyphenyl)glycine residue in polymastiamide A (**1**)[5] on the basis of the similarity in the ^1H- and ^{13}C-NMR chemical shifts of the amino acid α-carbon and α-proton resonances in the desulfated methyl ester derivatives of all the compounds.

Sterol **13** was obtained as a colorless solid that gave a parent ion at *m/z* 619.42404 in the HREIMS, appropriate for a molecular formula of $C_{39}H_{57}NO_5$, indicating that it was isomeric with polymastiamide A methyl ester (**7**). It was evident from a comparison of the ^1H- and ^{13}C-NMR data obtained for **13** and **7** (Tables 1 and 2) that both molecules had identical (*p*-methoxyphenyl)-glycine methyl ester and steroid side chain substructures and that they differed in the C/D ring portions of the steroid nucleus. A resonance observed at δ 5.08 (H15) in the ^1H-NMR spectrum of compound **13**, which was correlated into an olefinic methine carbon resonance at δ 116.9 (C15) in the HMQC spectrum, and the presence of a quaternary olefinic carbon resonance at δ 155.5 (C14) in the ^{13}C/APT-NMR spectrum indicated that the steroid nucleus of **13** contained a trisubstituted olefin. Detailed analysis of the COSY, HMQC, and APT data obtained for **13** identified all the proton resonances on the A and B rings providing an unambiguous assignment of the Me19 proton (δ 0.82) and carbon (δ 13.1) resonances (Tables 1 and 2). A strong HMBC correlation between the remaining methyl singlet proton resonance at δ 0.84, assigned to Me18, and the quaternary carbon resonance at δ 155.5 (C14) placed the trisubstituted olefin at either the $\Delta^{14,15}$ or $\Delta^{16,17}$ position. COSY correlations, which identified a contiguous proton spin system starting at the olefinic methine resonance at δ 5.08 (H15) and continuing through the H16/H16′, H17, H20, and Me21 resonances, located the trisubstituted olefin at the $\Delta^{14,15}$ position. An overlapping network of HMBC correlations observed between the olefinic proton H15 (δ 5.08) and C13 (δ 47.0), C16 (δ 35.5), and C17 (δ 58.4) resonances, and between one of the H16 allylic protons (δ 2.18) and the C13 (δ 47.0), C14 (δ 155.5), C15 (δ 116.9), and C17 (δ 58.4) resonances were completely consistent with the proposed D-ring constitution.

The relative configuration at C8 in **13** could be deduced from $^1H/^1H$ coupling constants. The splitting

Table 2. ^{1}H NMR Spectral Data (δ) in $CDCl_3$ at 500 MHz

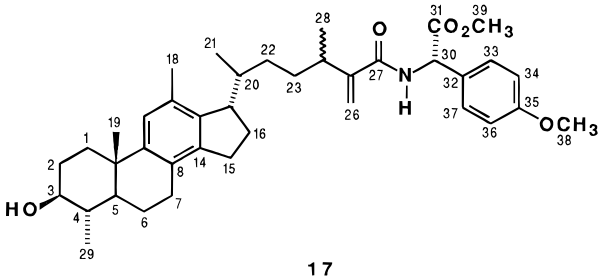
H no.	compound										
	7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1e	1.68	1.67, dt (13.8, 3.4)	1.68, dt (13, 3)	1.68	1.68	1.68	1.77	1.75	1.79	1.79	2.26, dt (13.5, 3.5)
1a	1.12	1.08	1.12	1.09	1.13	1.12	0.97, td (13.4, 4.3)	0.95, td (13.8, 4.3)	0.97, td (13.8, 4.3)	0.98, td (13.6, 4.2)	1.48
2e	1.81, dq (13, 4)	1.80	1.81	1.80	1.81, dq (13, 4)	1.81	1.79	1.78	1.79	1.78	1.96
2a	1.42	1.33	1.42	1.35	1.42	1.42	1.47	1.54	1.47	1.46	1.67, qd
3a	3.11, td (10.8, 4.4)	3.60, tt (10.0, 5.0)	3.10, td (10.5, 4.8)	3.60, td (10.8, 4.4)	3.11, td (10.8, 4.5)	3.10, td (10.5, 4.7)	3.06, ddd (11.1, 9.9, 4.3)	3.57, tt (10.8, 4.7)	3.06, td (10.5, 4.7)	3.06, ddd (10.8, 10.1, 4.6)	3.16, td (10.5, 4.3)
4e			1.61	1.61				1.57			
4a	1.26	1.25	1.26	1.24	1.26	1.26	1.28	1.27	1.29	1.30	1.41
5a	0.84	1.24	0.86	1.24	0.84	0.85	0.68, td (11.3, 2.9)	1.07, td (11.4, 2.9)	0.69, td (11.4, 2.9)	0.68, td (11.5, 2.6)	1.20, td
6e	1.69	1.33	1.69	1.33	1.69	1.69	1.71, dq (12.5, 3.3)	1.32	1.72, dq (12.7, 3.0)	1.71, dq (12.6, 2.9)	1.98
6a	0.98	1.19	0.98	1.20	0.97	0.98	1.07	1.32	1.08, qd (12.8, 3.1)	1.07, qd (12.6, 2.7)	1.48
7e	2.38, m	2.34, m	2.38, m	2.34, m	2.38	2.39, m	1.92, dq (13.8, 3.3)	1.87	1.94	1.93	2.64
7a	1.65	1.74	1.65	1.74	1.64	1.66	1.14	1.15	1.15	1.12	2.57
8							1.93	1.97	1.97	1.97	
9a	1.61	1.61	1.60	1.61	1.61	1.61	0.63, td (11.6, 2.8)	0.64, td (12.0, 2.8)	0.64, td (12.0, 2.8)	0.64, td (11.4, 2.6)	
11e	1.58	1.58	1.58	1.59	1.58	1.58	1.55	1.53	1.57	1.58	6.85, bs
11a	1.43	1.45	1.44	1.46	1.43	1.44	1.28	1.29	1.30	1.28	
12e	1.88, dt (12.2, 4)	1.88, dt (12.7, 3.6)	1.90, dt (12.5, 3.0)	1.91, dt (12.2, 4)	1.88, dt (12.2, 4)	1.90, dt (12.5, 3.5)	1.94, dt (12.6, 3.1)	1.94, dt (12.8, 3.2)	1.97, dt (12.7, 3.2)	1.97, dt (12.5, 3.2)	
12a	1.06	1.08	1.08, td (13.2, 3.5)	1.09	1.06	1.09	1.16	1.18	1.20	1.21	
15	2.16	2.17, m	2.20	2.20	2.16	2.22	5.08, bs	5.08, bs	5.12, bs	5.12, bs	2.67
15′	2.16	2.14, m	2.18	2.20	2.16	2.18					2.54
16	1.73	1.73	1.79	1.80	1.72	1.80	2.18, ddd (15.8, 9.6, 2.2)	2.17, ddd (15.4, 7.5, 1.9)	2.25, ddd (15.3, 7.7, 2.9)	2.25, ddd (15.2, 7.4, 2.3)	1.88
16′	1.27	1.27	1.35	1.33	1.27	1.35	1.79	1.77	1.87	1.89	1.88
17	1.06	1.06	1.12	1.12	1.06	1.13	1.43	1.44	1.50	1.51	3.22, dt
18	0.78, s	0.78, s	0.81, s	0.81, s	0.78	0.81, s	0.84, s	0.87, s	0.87, s	0.88, s	2.19, bs
19	0.69, s	0.66, s	0.69, s	0.66, s	0.69	0.69, s	0.82, s	0.82, s	0.82, s	0.82, s	1.11, s
20	1.37	1.37	1.49	1.49	1.37	1.50	1.50	1.51	1.62	1.61	1.87
21	0.86, d (6.6)	0.86, d (6.6)	0.94, d (6.6)	0.94, d (6.6)	0.85, d (6.7)	0.94, d (6.6)	0.835, d (6.4)	0.835, d (6.4)	0.92, d (6.6)	0.92, d (6.6)	0.49, d (6.6)
22	1.36	1.36	1.51	1.52	1.36	1.52	1.34, m	1.35	1.51	1.50	1.38
22′	1.01	1.01	1.20	1.20	1.01	1.20	0.96	0.96	1.17	1.15	1.23
23	1.52	1.50	2.17, m	2.17	1.50	2.17	1.53	1.53	2.18, m	2.18, m	1.56
23′	1.17	1.18	2.01, m	2.01, m	1.16	2.02, m	1.18	1.19	2.03, m	2.05, m	1.33
24	2.57, dt (6.8)	2.57, dt (6.8)	6.38, t (6.7)	6.38, bt (6.7)	2.57, dt (6.9, 6.9)	6.40, t (6.7)	2.58, dt (6.6)	2.58, dt (6.7)	6.38, t (6.7)	6.40, t (7.6)	2.65
26	5.57, bs	5.57, bs	1.83, bs	1.83, bs	5.58, bs	1.84, bs	5.57, bs	5.57, bs	1.84, bs	1.85, bs	5.57, bs
26′	5.22, bs	5.22, bs			5.23, bs		5.23, bs	5.23, bs			5.25, bs
28	1.05, d (6.8)	1.05, d (6.8)			1.05, d (6.8)		1.05, d (6.9)	1.05, d (6.9)			1.08, d (7)
29	0.97, d (6.3)		0.96, d (6.3)		0.97, d (6.4)	0.97, d (6.3)	0.95, d (6.3)		0.95, d (6.3)	0.95, d (6.3)	1.05, d (6.3)
NH	6.65, d (7.1)	6.65, d (7.2)	6.59, d (6.8)	6.58, d (6.8)	6.72, d (7.1)	6.65, d (7.0)	6.67, d (7.1)	6.67, d (7.1)	6.59, d (6.8)	6.65, d (7.0)	6.59, d (6.8)
30	5.55, d (7.1)	5.55, d (7.2)	5.53, d (6.8)	5.54, d (6.8)	5.61, d (7.1)	5.61, d (7.0)	5.54, d (7.1)	5.54, d (7.1)	5.54, d (6.8)	5.61, d (7.0)	5.54, d (6.8)
33	7.27, d (8.7)	7.27, d (8.8)	7.28, d (8.7)	7.28, d (8.7)	7.34	7.35	7.27, d (8.8)	7.27, d (8.7)	7.29, d (8.6)	7.37	7.28, d (8.7)
34	6.85, d (8.7)	6.85, d (8.8)	6.86, d (8.7)	6.86, d (8.7)	7.35	7.36	6.85, d (8.8)	6.85, d (8.7)	6.86, d (8.6)	7.35	6.84, d (8.7)
35					7.31	7.32				7.33	
36	6.85, d (8.7)	6.85, d (8.8)	6.86, d (8.7)	6.86, d (8.7)	7.35	7.36	6.85, d (8.8)	6.85, d (8.7)	6.86, d (8.6)	7.35	6.84, d (8.7)
37	7.27, d (8.7)	7.27, d (8.8)	7.28, d (8.7)	7.28, d (8.7)	7.34	7.35	7.27, d (8.8)	7.27, d (8.7)	7.29, d (8.6)	7.37	7.28, d (8.7)
38	3.77, s	3.77, s	3.77, s	3.77, s			3.77, s	3.77, s	3.77, s		3.75, s
39	3.72, s	3.72, s	3.71, s	3.72, s	3.73	3.72, s	3.72, s	3.72, s	3.71, s	3.72, s	3.72, s

pattern and *J* values observed for the H9 resonance (δ 0.63, td, J = 11.6, 2.8 Hz) defined its axial orientation and further required that there exist two vicinal axial ($H8_{ax}$, δ 1.93 and $H11_{ax}$, δ 1.28) and one vicinal equatorial ($H11_{eq}$, δ 1.55) coupling partners. Therefore, it was apparent that compound **13** differed from methyl ester **7** only in the placement of the double bond on the steroid nucleus.

^{1}H-NMR screening of the crude sponge extracts and the fractions eluted from Sephadex LH-20 chromatography revealed that none of the naturally occurring polymastiamides gave ^{1}H NMR resonances at δ 5.1 that could be assigned to the H15 olefinic proton found in compound **13**. Therefore, it appeared that sterol **13** was most likely an artifact formed from the natural product polymastiamide A (**1**) during the reaction with MeOH/HCl. Support for the artifact hypothesis came from the treatment of pure polymastiamide A methyl ester (**7**) with the standard methylation conditions (MeOH/HCl 12 h, 40 °C), which gave a mixture of the isomerized product **13** and the unreacted methyl ester **7**.

Sterols **14**, **15**, and **16** were obtained as colorless solids. Sterol **14** gave a parent ion in the HREIMS at *m/z* 605.40936, appropriate for a molecular formula of $C_{38}H_{55}NO_5$; **15** gave a parent ion in the HREIMS at *m/z* 605.40839, appropriate for a molecular formula of $C_{38}H_{55}NO_5$; and **16** gave a parent ion in the HREIMS at *m/z* 575.39762, appropriate for a molecular formula of $C_{37}H_{53}NO_4$. All three compounds showed a broad deshielded resonance at δ 5.1 ppm in the ^{1}H-NMR spectrum that could be assigned to an H15 olefinic methine as discussed above for **13**. Detailed analysis of the 1D and 2D NMR data for these three compounds showed that sterols **14**, **15**, and **16** were the rearranged products of polymastiamides B (**2**), C (**3**), and F (**6**), respectively (Tables 1 and 2).

Sterol **17** was obtained as a colorless solid. It gave a



17

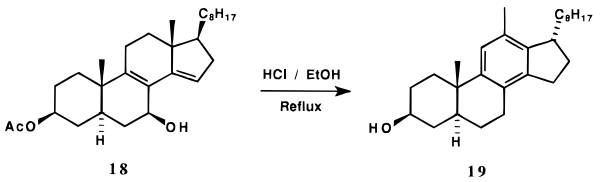
parent ion in the HREIMS at *m/z* 615.39208, appropriate for a molecular formula of $C_{39}H_{53}NO_5$, differing from that of polymastiamide A methyl ester (**7**) only by the lack of four hydrogen atoms. Comparison of the ^{1}H- and ^{13}C-NMR data for **17** with the corresponding data for **7** showed that they contained identical *p*-methoxyphenylglycine methyl ester fragments. Furthermore, analysis of the ^{13}C, COSY, and HMBC data showed that the side chain in steroid **17** had a constitution identical to the side chain in steroid **7** (Tables 1 and 2).

Once the nature of the side chain and the (*p*-methoxyphenyl)glycine methyl ester fragments were established, the remaining portion of **17** had to account for a composition of $C_{20}H_{27}O$, which required seven sites of unsaturation. Because there were 16 sp^2 carbons in the ^{13}C-NMR spectrum of **17**, 10 of which were assigned to the steroid side chain and *p*-methoxyphenylglycine methyl ester fragment, the steroid nucleus had to contain six sp^2 carbons. The chemical shift and scalar couplings of the H3 carbinol methine proton (δ 3.16, td, J = 10.5, 4.3 Hz) suggested that it had an axial orientation and was shielded by the equatorial methyl group at C4 (Me29, δ 1.05) as discussed previously for **7**. COSY correlations between a methyl doublet at δ 1.05 (Me29) and a methine proton at δ 1.41 (H4), which showed additional COSY correlations to the H3 resonance at δ 3.16 as well as to the H5 resonance at δ 1.20, confirmed the presence of a methyl substituent (Me29) at C4. Further COSY correlations were observed between H5 (δ 1.20) and $H6_{eq}$ (δ 1.98), and between $H6_{eq}$/$H6_{ax}$ (δ 1.98/ 1.48) and H7/H7′ (δ 2.64/ 2.57). The carbinol methine proton H3 (δ 3.16) was also correlated to a pair of methylene proton resonances at δ 1.96/ 1.67, assigned to $H2_{eq}$/$H2_{ax}$, and the latter exhibited further correlations to resonances at δ 2.26/ 1.48, assigned to $H1_{eq}$/$H1_{ax}$. Analysis of the HMBC data provided support for the proton assignments for the A/B rings. The ^{1}H methyl doublet at δ 1.05 (Me29) showed strong HMBC correlations to a ^{13}C carbinol resonance at δ 76.5 (C3) and to two ^{13}C methine carbon resonances at δ 39.5 (C4) and 46.5 (C5). The C5 resonance at δ 46.5 was also correlated in the HMBC spectrum to a ^{1}H methyl singlet resonance at δ 1.11, which was assigned to Me19. The methyl singlet (Me19, δ 1.11) showed additional HMBC correlations to C1 and C10 (both δ 36.5).

The presence in the ^{1}H-NMR spectrum of **17** of an aromatic resonance at δ 6.85 (H11) and a broad singlet integrating for three protons at δ 2.19 (Me18), assigned to a benzylic methyl group, indicated that the steroid nucleus contained a benzene ring. Because the aliphatic nature of the A and B rings had already been established, it was apparent that ring C had to be aromatic. The benzylic methyl resonance (δ 2.19; Me18) showed HMBC correlations to two non-protonated sp^2 carbon resonances at δ 130.4 (C12) and 141.9 (C13) and to the aromatic methine carbon at δ 124.5 (C11), which was correlated in the HMQC spectrum into the ^{1}H resonance at δ 6.85. Thus, the benzylic methyl and aromatic proton had to be ortho to each other. An HMBC correlation observed between the aromatic proton H11 (δ 6.85) and the aliphatic quaternary carbon C10 (δ 36.5) located the aromatic proton at C11 and, consequently, the aromatic methyl at C12 to give the overall structure **17**. Confirmation of the proposed D ring substructure in **17** came from analysis of the COSY data. A deshielded methine proton resonance at δ 3.22 (H17), which was coupled to a carbon at δ 48.2 (C17) in the HMQC spectrum, showed COSY correlations to H16/H16′ (both δ 1.88), and the latter resonances were further correlated to H15/H15′ (δ 2.67/ 2.54). Finally, the α stereochemistry of C17 was established by comparison of ^{1}H-NMR chemical shifts assigned to H17 and Me21 in **17** with the literature values for the C-ring benzenoid steroid **19** (17 H17, δ 3.22; Me21, δ 0.49: **19** H17, δ 3.30; Me21, δ 0.55).[7] The base peak in both the LREIMS and HREIMS of **17** at *m/z* 283.20643 was appropriate for the steroid nucleus ion of $C_{20}H_{27}O$ resulting from loss of the side chain by a facile benzylic cleavage.

Steroids with aromatic C rings are extremely rare. There appear to be no naturally occurring examples reported to date. All C-ring benzenoid steroids in the literature were prepared by the aromatization of hy-

Scheme 1



roxylated steroid precursors. For example, the treatment of 3β-acetoxy-5α-cholesta-8,14-dien-7β-ol (**18**) with HCl in refluxing EtOH gave the aromatic sterol **19** in good yield (Scheme 1). The side-chain configuration (C17) was inverted in the aromatization of **18** promoted by HCl.[7]

Because HCl/MeOH was utilized in the derivatization of the polymastiamide mixture, sterol **17** obtained in our investigation was very likely a rearrangement artifact. The natural product that leads to sterol **17** is not known at present; however, it is certain that the polymastiamide A (**1**) could not be the precursor of sterol **17** as the two compounds have different oxidation states. Our observation that the reaction of pure methyl ester **7** with MeOH/HCl under the methylation conditions (12 h, 40 °C) did not give any trace of sterol **17** is consistent with the above hypothesis.

Steroids isolated from marine sponges frequently contain substantially modified side chains.[1−4] The interesting side-chain modification in the polymastiamide series (**1**−**6**), which involves the linkage to a nonprotein amino acid via an amide bond, represents an interesting new type of steroid modification.

Experimental Section

General Experimental Procedures. LREIMS and HREIMS were recorded on a Kratos MS50/DS55SM mass spectrometer. Gel permeation chromatography was performed using Sephadex LH-20 resin. Normal-phase column chromatography was carried out on either Merck Si gel G60 (230−400 mesh) or Sigma Si gel (size: 10−40 μ). HPLC was performed on Waters instruments (996, 486, 440) with a normal-phase Si gel column packed with 8MP10μ using UV as a detector. TLC was conducted on precoated Kieselgel 60 F_{254} (Art 5554; Merck), and the spots were detected by UV and/or heating after spraying with vanillin reagent. NMR spectra were recorded on Bruker spectrometers (WH-400 and AMX-500) at either 500 MHz or 400 MHz (^{1}H) and 125 MHz (^{13}C). The chemical shifts are reported in ppm downfield from the TMS resonance with the solvent residual peaks as the references (^{1}H: $CDCl_3$, 7.24 ppm; ^{13}C: $CDCl_3$, 77.0 ppm). The coupling constants (J) are given in Hz.

Material. Specimens of *P. boletiformis* were collected by hand using SCUBA at depths of 20−25 m on vertical rock faces off Korsnes Peninsula on Fanafjiord, south of Bergen, Norway, in July 1992. Freshly collected sponge (2 kg wet wt) was frozen on site, transported to Vancouver over dry ice, and stored in a freezer. The organisms had a firm-textured body with raised oscula on the surface. They were bright orange and round, and up to 12 cm in diameter and thickness. The sponge was identified by Prof. R. van Soest, A voucher sample is deposited at the Zoological Museum of Amsterdam (ZMA POR 10170).

Isolation. A portion of the frozen sponge (152 g) was chopped, immersed in MeOH, and soaked at room temperature for 2 days. The MeOH extract was concentrated *in vacuo* to give a red suspension that was partitioned between an aqueous solution (300 mL) of H_2O−MeOH 1:1 and hexanes (200 mL × 2). The aqueous layer was concentrated and lyophilized to yield a brown solid (12.2 g, mostly salt). The solid was suspended in MeOH, and the suspension was then filtered. The filtrate was concentrated *in vacuo* and chromatographed on Sephadex LH-20 (eluent: MeOH) to give a fraction containing steroidal compounds (378 mg). This fraction was desalted by reversed-phase silica column chromatography (eluent: first H_2O, then H_2O−MeOH 50:50) to afford 193 mg of steroid mixture. Repeated chromatography on Sephadex LH-20 (eluent: EtOAc−MeOH−H_2O 40:10:4) yielded pure polymastiamide A (**1**) (34 mg) and a mixture of the other closely related analogues.

Methylation and Separation. All fractions containing polymastiamide analogues were combined and treated with 2 N HCl acid in MeOH−H_2O (1:1) solution at 50 °C for 4 h. The reaction mixture was evaporated *in vacuo* and partitioned between EtOAc and H_2O. The EtOAc layer was concentrated and chromatographed on a normal-phase Si gel column (eluent: EtOAc−Hexane 30:70) to give crude products. Repeated fractionation of the crude products via normal-phase HPLC (eluent: a. EtOAc−hexane 25:75 to 50:50; b. EtOAc−CH_2Cl_2 8:92) led to the separation and purification of methyl esters **11** and **12**, sterol **16**, methyl ester **7**, sterol **13**, sterol **17**, methyl ester **9**, sterol **15**, methyl ester **8**, sterol **14**, and methyl ester **10**, in elution sequence.

Polymastiamide B methyl ester (8): colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS m/z (% rel int) 605 (2.9), 587 (2.4), 546 (1.6), 194 (98), 179 (100); HREIMS m/z $[M]^+$ 605.40806 ($C_{38}H_{55}NO_5$ ΔM +0.0 mmu).

Polymastiamide C methyl ester (9): colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS m/z (% rel int) 605 (14.9), 587 (5.8), 572 (3.6), 546 (3.2), 194 (100), 179 (74); HREIMS m/z $[M]^+$ 605.40820 ($C_{38}H_{55}NO_5$ ΔM +0.2 mmu).

Polymastiamide D methyl ester (10): colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS m/z (% rel int) 591 (12.8), 573 (3.4), 559 (2.7), 532 (2.9), 297 (2.3), 194 (100), 179 (74.8); HREIMS m/z $[M]^+$ 591.39203 ($C_{37}H_{53}NO_5$ ΔM −0.4 mmu).

Polymastiamide E methyl ester (11): colorless solid; ^{1}H NMR see Table 2; LREIMS m/z (% rel int) 589 (18.5), 571 (28), 556 (36), 285 (21), 283 (29), 166 (100), 149 (27); HREIMS $[M]^+$ m/z 589.41223 ($C_{38}H_{55}NO_4$ ΔM −0.9 mmu).

Polymastiamide F methyl ester (12): colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS m/z (% rel int) 575 (93), 557 (54), 542 (52), 516 (14), 410 (21), 392 (22), 382 (34), 367 (23), 283 (81), 260 (55), 247 (41), 221 (68), 166 (78), 164 (48), 149 (44); HREIMS m/z $[M]^+$ 575.39667 ($C_{37}H_{53}NO_4$ ΔM −0.8 mmu).

Sterol 13: colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS m/z (% rel int) 619 (9.4), 601 (1.9), 587 (1.4), 560 (2.0), 285 (16), 194 (100), 179 (65); HREIMS m/z $[M]^+$ 619.42404 ($C_{39}H_{57}NO_5$ ΔM +0.4 mmu).

Sterol 14: colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS *m/z* (% rel int) 605 (7.7), 587 (1.7), 573 (1.7), 546 (3.0), 426 (2.2), 410 (2.4), 382 (2.4), 299 (7.7), 271 (16), 255 (8.1), 194 (100), 179 (59); HREIMS *m/z* $[M]^+$ 605.40936 ($C_{38}H_{55}NO_5$ ΔM +1.3 mmu).

Sterol 15: colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS *m/z* (% rel int) 605 (14), 587 (1.7), 573 (0.9), 546 (2.5), 410 (10.3), 383 (2.9), 355 (7.2), 329 (5.3), 313 (12), 302 (27.6), 285 (19.4), 251 (11.7), 194 (93), 179 (100); HREIMS *m/z* $[M]^+$ 605.40839 ($C_{38}H_{55}NO_5$ ΔM +0.4 mmu).

Sterol 16: colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS *m/z* (% rel int) 575 (2.6), 557 (0.9), 542 (1.7), 516 (3.1), 499 (1.3), 410 (27.2), 383 (9.8), 367 (7.7), 329 (8.4), 313 (25), 285 (46), 260 (46), 221 (29), 163 (37), 149 (27); HREIMS *m/z* $[M]^+$ 575.39762 ($C_{37}H_{53}NO_4$ ΔM +0.2 mmu).

Sterol 17: colorless solid; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1; LREIMS *m/z* (% rel int) 615 (8.6), 597 (0.8), 582 (1.0), 556 (0.8), 283 (100), 194 (22), 179 (32), 143 (15.2); HREIMS *m/z* $[M]^+$ 615.39208 ($C_{39}H_{53}NO_5$ ΔM −0.3 mmu).

Acknowledgment. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada. The authors wish to thank M. Le Blanc (UBC) and Dr. U. Bamstedt (University of Bergen) for assisting with the collection of *P. boletiformis*.

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NP960098O