New Geodiamolides from the Sponge *Cymbastela* sp. Collected in Papua New Guinea

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Geodiamolides J−P (**11**−**17**) and R (**19**), eight new cyclic depsipetides, have been isolated from the marine sponge *Cymbastela* sp. collected in Papua New Guinea. The serine residue in geodiamolides L−P (**13**−**17**) and R (**19**) has not been previously found in this family of compounds.

Two families of closely related cyclic depsipeptides, the jaspamides and the geodiamolides, have been isolated from a variety of tropical marine sponges. Jaspamide (jasplaki-nolide) (**1**), obtained independently from *Jaspis* sp. collected in Palau[1] and Fiji,[2] was the first member of this group of depsipetides to be reported. Shortly thereafter, geodi-amolides A (**2**) and B (**3**) were isolated from the Caribbean sponge *Geodia* sp.[3] Subsequently, geodiamolides C−G (**4**−**8**)[4,5] have been reported from a *Cymbastela* sp. collected in Papua New Guinea; geodiamolides H (**9**) and I (**10**) have been reported from a *Geodia* sp.;[6] geodiamolide TA (**20**) has been reported from the South African sponge *Hemiastrella minor*;[7] and neosiphoniamolide A (**21**) has been reported from the New Caledonian sponge *Neosiphonia superstes.*[8] There has been considerable ongoing interest in the total synthesis[9,10] and drug potential of the jaspamides and geodiamolides as a result of their novel structures and potent cytotoxic,[5,11,12] antifungal,[3] and insecticidal activ-ites.[2] Recently, we isolated the new antimitotic linear peptides hemiasterlins A and B, and criamides A and B from specimens of the same Papua New Guinea *Cym-bastela* sp. (family Axinellidae, order Halichondrida) that earlier yielded geodiamolides C−G (**4**−**8**).[5] This recollected *Cymbastela* sp. sample also yielded small amounts of eight new cyclic depsipetides, geodiamolides J−P (**11**−**17**), and R (**19**), whose structures are reported herein. Geodi-amolides L−P (**13**−**17**) and R (**19**) represent the first examples in this class of compounds in which a serine residue has been incorporated into the structure (Chart 1).

Results and Discussion

To obtain sufficient amounts of the hemiasterlins and criamides for in vivo evaluation of their cytotoxic activities, the *Cymbastela* sp. was recollected from the original dive sites at Motupore and Madang in Papua New Guinea. Bioassay-guided fractionation of extracts from the recol-lected *Cymbastela* specimens led to the isolation of hemi-asterlins and criamides,[5] the known geodiamolides A−G (**2**−**8**),[3−5] and the novel geodiamolides J−P (**11**−**17**) and R (**19**).

Geodiamolides J (**11**) and K (**12**) gave parent ion clusters in the HRDCIMS, corresponding to molecular formulas of $C_{28}H_{39}N_3O_7Br$ [610.19431/608.19508 (ΔM −1.49, −3.38 ppm)], and $C_{28}H_{39}N_3O_7Cl$ [565.23956/563.24011 (ΔM −4.7,

Table 1. ^{1}H NMR Data for Geodiamolides G (**8**), J (**11**), and K (**12**) in $CDCl_3$ at 500 MHz (*J* in Hz)

position	**8** δ_H	**11** δ_H	**12** δ_H
2	2.46 m	2.45 m	2.46 m
3	2.55 dd (4, 12)	2.54 dd (4, 13)	2.54 d (12)
3′	2.14 t (12)	2.14 (12)	ns[a]
6	2.95	ns[a]	ns[a]
7	1.82 ddd (3, 9, 15)	1.82 m	ns[a]
7′	1.61 ddd (3, 11, 15)	1.60 m	ns[a]
8	5.11 m	5.11 m	5.11 m
10	4.51 dq (7, 7)	4.50 dq (7, 7)	4.51 m
NH(10)	6.35 d (7)	6.21 d (7)	6.21 d (8)
12	5.06 dd (8, 9)	5.06 dd (8, 9)	5.06 m
14	4.72 dq (7)	4.71 dq (7, 8)	4.71 m
NH(14)	6.19 d (7)	6.36 d (8)	6.37 d (8)
15	3.12 dd (8, 15)	3.13 dd (8, 15)	3.14 m
15′	2.90 dd (9, 15)	2.91 dd (9, 15)	2.90 m
17	7.45 d (1)	7.25 d (2)	7.12 s
20	6.88 d (8)	6.91 d (8)	6.91 d (8)
21	7.04 dd (1, 8)	7.02 dd (2, 8)	6.98 d (8)
22	1.15 d (6)	1.14 d (7)	1.14 d (7)
23	5.90 s	5.90 s	5.90 s
23′	5.78 s	5.78 s	5.79 s
24	1.09 d (7)	1.09 d (7)	1.09 d (7)
25	1.28 d (6)	1.28 d (6)	1.28 d (6)
26	1.32 d (7)	1.31 d (7)	1.31 d (7)
27	2.97 s	2.96 s	2.96 s
28	1.04 d (7)	1.03 d (7)	1.03 d (7)

[a] Signals not seen due to sample size or interference with H_2O peak or both.

−0.5 ppm)], respectively. A comparison of the ^{1}H NMR data for **11** and **12** with the ^{1}H NMR data for geodiamolide G (**8**) (Table 1) revealed that the resonances for the alanine−polyketide−alanine portions of the molecules (H-1 to H-8, H-12 to H-20, H-22 to H-25, and H-27) were identical, and the only significant difference was observed in the chemical shifts of the resonances assigned to H-17 in the tyrosine residue. The upfield shift of H-17 from δ 7.45 in geo-diamolide G (**8**) to δ 7.25 in **11** and to δ 7.12 in **12** was analogous to the observed changes in the chemical shift of H-17 in geodiamolides B (**3**) and C (**4**) relative to geodi-amolide A (**2**), where the C-18 substituent varies from iodine in **2** to bromine in **3** and chlorine in **4**.[3,4] Thus, geodiamolides J (**11**) and K (**12**) were the C-18 bromo and chloro analogues of geodiamolide G (**8**), respectively.
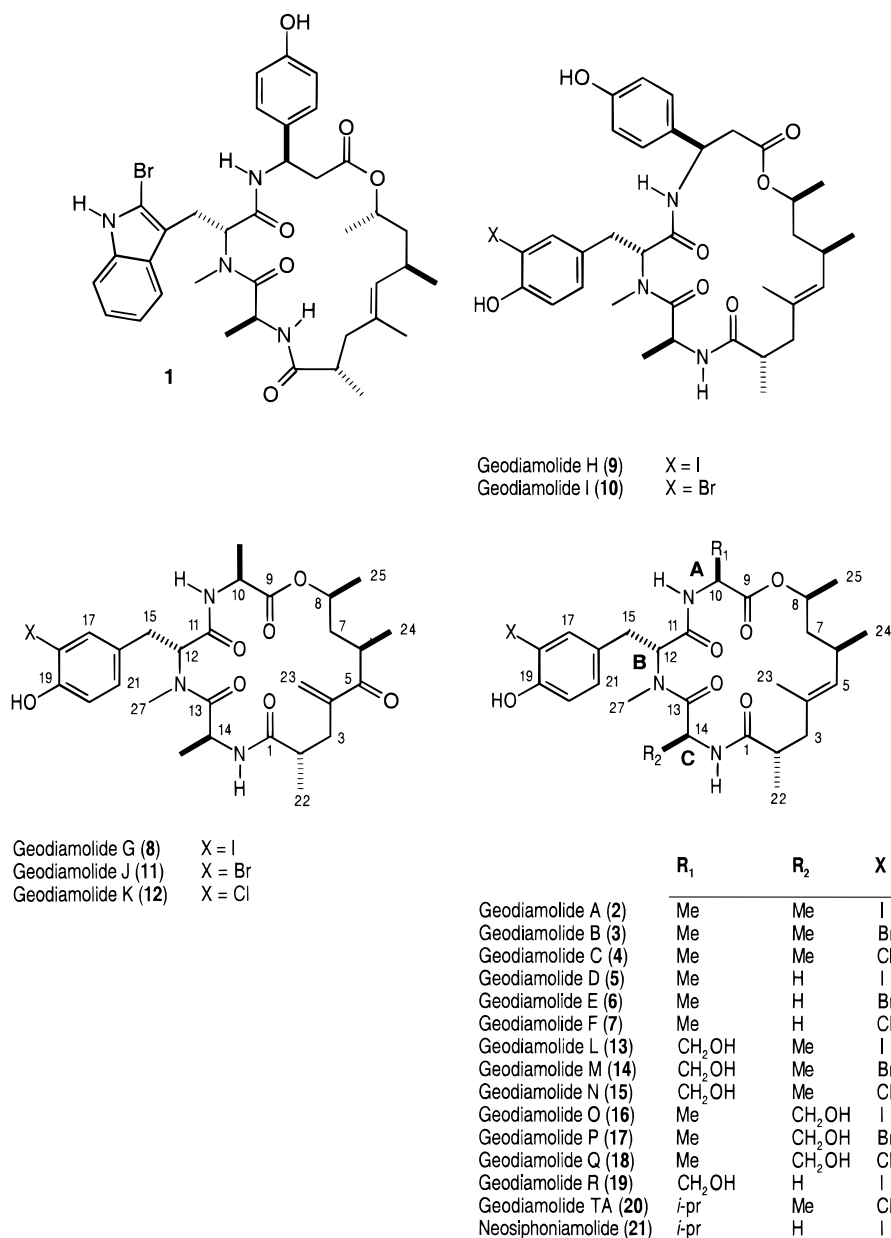
Geodiamolide L (**13**) gave a M[+] ion in the HREIMS at *m*/*z* 658.19767, appropriate for a molecular formula of $C_{28}H_{40}O_7N_3I$ (ΔM −1.91 ppm). The molecular formula of **13** differed from that of geodiamolide A (**2**) simply by the addition of one oxygen atom, suggesting the addition of a

Chart 1

Geodiamolide H (**9**)   X = I
Geodiamolide I (**10**)   X = Br

Geodiamolide G (**8**)   X = I
Geodiamolide J (**11**)   X = Br
Geodiamolide K (**12**)   X = Cl

	R_1	R_2	X
Geodiamolide A (**2**)	Me	Me	I
Geodiamolide B (**3**)	Me	Me	Br
Geodiamolide C (**4**)	Me	Me	Cl
Geodiamolide D (**5**)	Me	H	I
Geodiamolide E (**6**)	Me	H	Br
Geodiamolide F (**7**)	Me	H	Cl
Geodiamolide L (**13**)	CH_2OH	Me	I
Geodiamolide M (**14**)	CH_2OH	Me	Br
Geodiamolide N (**15**)	CH_2OH	Me	Cl
Geodiamolide O (**16**)	Me	CH_2OH	I
Geodiamolide P (**17**)	Me	CH_2OH	Br
Geodiamolide Q (**18**)	Me	CH_2OH	Cl
Geodiamolide R (**19**)	CH_2OH	H	I
Geodiamolide TA (**20**)	*i*-pr	Me	Cl
Neosiphoniamolide (**21**)	*i*-pr	H	I

hydroxyl group. Detailed analysis of the ^{1}H NMR (Table 2), COSY, HMQC, and HMBC spectra of **13** showed that it contained an alanine residue and the same polyketide and *N*-methyl iodotyrosine moieties found in geodiamolide A (**2**). The COSY data also identified an isolated spin system containing a N*H* resonance at δ 7.00 (d, $J = 8$ Hz: N*H*-10), an amino acid α-methine resonance at δ 4.43 (m: H-10), and a pair of diastereotopic methylene proton resonances at δ 3.97 (dd, $J = 11, 4$ Hz: H-26) and δ 3.81 (dd, $J = 11, 4$ Hz: H-26'). HMQC data showed that the latter protons were attached to a carbon with a chemical shift of δ 63.1 (C-26), and an HMBC correlation from the α-methine resonance at δ 4.43 (H-10) to a carbonyl resonance at δ 169.8 (C-9) confirmed the presence of a serine residue. Thus, **13** was simply a serine analogue of geodiamolide A (**2**). HMBC correlations observed between the resonances assigned to the iodotyrosine *N*-Me (δ 2.97, s: Me-27) and alanine carbonyl C-13 (δ 174.6), and between the alanine N*H* resonance at δ 6.44 (d, $J = 6$ Hz: N*H*-14) and the polyketide carbonyl resonance (δ 175.5: C-1) situated the alanine residue between the *N*-Me-iodo-

tyrosine and polyketide moieties. An ion observed at *m/z* 409 Da in the MS/MS electrospray mass spectrum of the molecular ion of geodiamolide L (**13**) was attributed to a protonated tyrosine–serine dipeptide, which results from the cleavage C-13/N-12 amide bond and the cleavage of the C-8/O bond via a McLafferty rearrangement involving H-7. The observation of this fragment indicated that the serine residue must be attached to the amide carbonyl carbon (C-11) of the methyl iodotyrosine through an amide bond and to the polyketide fragment via an ester linkage, thus completing the assignment of the planar structure of geodiamolide L (**13**).

Geodiamolides M (**14**) and N (**15**) gave parent ions in the HREIMS consistent with molecular formulas of $C_{28}H_{39}N_3O_7Br$ [611.20371/609.20519 (ΔM $-1.3/-0.4$ ppm)] and $C_{28}H_{39}N_3O_7Cl$ [567.25184/565.25403 (ΔM 1.2/2.6 ppm)], respectively. The ^{1}H NMR data for **14** and **15** (Table 2) were almost identical to the data obtained for geodiamolide L (**13**), with the exception of the chemical shifts assigned to the H-17 resonances in the tyrosine moiety. The chemical shift of H-17 varied from δ 7.46 in geodiamolide L (**13**) to

Table 2. NMR Data for Geodiamolides A (**2**),3 L (**13**), M (**14**), N (**15**), O (**16**), and P (**17**) in CDCl$_3$ at 500 MHz (*J* in Hz)

position	**2** δ_C	**2** δ_H	**13** $\delta_C{}^a$	**13** δ_H	**14** δ_H	**15** δ_H	**16** $\delta_C{}^a$	**16** δ_H	**17** δ_H
1	170.8		175.5				177.6		
2	42.2	2.32 m	42.4	2.26 m	2.28 m	2.27 m	42.2	2.40 m	2.41 m
3	43.3	2.16 d	42.3	2.17 m	2.17 m	2.16 m	43.2	2.14 m	2.10 m
3′		2.04 dd (4, 14)		1.98 dd (3, 14)	1.98 dd (3, 14)	1.98 dd (2, 12)		2.04 m	2.04 m
4	130.2		132.9				147		
5	131.6	4.93 d (9)	131.3	4.87 d (9)	4.87 d (9)	4.87 d (9)	131.2	4.93 d (9)	4.93 d (9)
6	28.9	2.16 m	29.3	2.18 m	2.18 m	2.16 m	28.7	2.16 m	2.15 m
7	43.5	1.63 ddd	43.7	1.63 ddd (6, 8, 14)	1.64 m	ns^b	43.9	1.56 m	1.56 m
7′		1.36 m		1.39 ddd (4, 8, 14)	1.37 m	1.37 m		1.37 m	1.37 m
8	70.9	4.88 m	71.9	4.94 m	4.94 m	4.93 m	70.6	4.87 m	4.87 m
9	168.7		169.8				170.4		
10	49.1	4.48 dq (7, 8)	53.3	4.43 dq (7, 7)	4.43 m	4.43 m	48.8	4.5 dq (7, 8)	4.45 m
NH(10)		6.54 d (8)		7.00 d (8)	6.99 d (8)	6.98 d (7)		6.45 d (7)	6.46 d (8)
11	175.3		168.9			168.4			
12	56.6	5.20 dd (8, 9)	56.9	5.26 dd (7, 9)	5.27 dd (7, 9)	5.28 dd (7, 9)	57.1	5.16 dd (8, 9)	5.17 dd (7, l0)
13	174.5		174.6				171.3		
14	45.8	4.75 dq (6, 7)	45.8	4.71 dq (4, 9)	4.71 quint (7)	4.72 quint (6)	52.8	4.71 m	4.79 m
NH(14)		6.47 d (6)		6.44 d (6)	6.43 d (6)	6.44 d (6)		6.67 br s	6.68 br s
15	32.4	3.16 dd (8, 15)	32.5	3.19 dd (7, 15)	3.22 dd (7, 15)	3.22 dd (7, 15)	32.2	3.17 dd (8, 15)	3.18 dd (7, 15)
15′		2.91 dd (9, 15)		2.89 dd (9, 15)	2.91 dd (9, 15)	2.93 dd (9, 15)		2.88 dd (9, 15)	2.91 dd (10, 15)
16	132.9		130.3				130.1		
17	138.3	7.48 d (2)	138.2	7.46 d (2)	7.27 d (2)	7.13 d (2)	138	7.46 d (2)	7.27 d (2)
18	85.2		85				85.3		
19	154.1		154.0				154.1		
20	115.0	6.89 d (8)	115.1	6.86 d (8)	6.91 d (8)	6.91 d (8)	114.9	6.88 d (8)	6.92 d (8)
21	130.4	7.07 dd (2, 8)	130.4	7.03 dd (2, 8)	7.03 dd (2, 8)	7.00 dd (2, 8)	130.1	7.05 dd (2, 8)	7.03 dd (2, 8)
22	18.73	1.15 d (7)	19	1.12 d (7)	1.12 d (7)	1.13 d (7)	18.8	1.16 d (7)	1.16 d (7)
23	17.58	1.50 d (1)	17.8	1.45 s	1.45 s	1.45 s	17.7	1.49 s	1.49 s
24	20.3	0.88 d (7)	20.5	0.86 d (7)	0.86 d (7)	0.87 d (7)	20.4	0.88 d (7)	0.86 d (6)
25	20.6	1.24 d (6)	20.8	1.24 d (6)	1.23 d (6)	1.23 d (6)	20.5	1.23 d (6)	1.23 d (6)
26	18.2	1.35 (7)	63.1	3.97 dd (4, 11)	3.97 dd (4, 11)	3.97 dd (4, 11)	18.1	1.32 d (7)	1.32 d (7)
26′				3.81 dd (4, 11)	3.82 dm (4, 11)	3.82 dm (4, 11)			
27	30.6	2.97	30.7	2.97	2.97 s	2.97 s	30.9	3.03 s	3.03 s
28	18.6	1.09 d (7)	18.5	1.08 d (7)	1.09 d (7)	1.09 d (7)	65.2	3.54 br s	3.54 br s
O*H*		5.44 br s							

^{a} Obtained from HMQC and HMBC spectra only. ^{b} Signals not seen.

δ 7.27 in **14** and δ 7.13 in **15**, consistent with **14** being the C-18 bromo analogue and **15** being the C-18 chloro analogue of geodiamolide L (**13**).

Geodiamolide O (**16**) gave a parent ion in the HREIMS at *m/z* M$^+$ 658.19880, appropriate for a molecular formula of C$_{28}$H$_{40}$O$_7$N$_3$I (ΔM −0.19 ppm). The molecular formula of geodiamolide O (**16**) was identical with the molecular formula obtained for geodiamolide L (**13**), and their ^{1}H NMR data were very similar. Analysis of the ^{1}H NMR (Table 2), COSY, HMQC, and HMBC spectra of **16** revealed that it contained the alanine, serine, *N*-methyl iodotyrosine, and polyketide fragments found in geodiamolide L (**13**). Unfortunately, the HMBC data obtained for **16** lacked correlations that would locate the serine and alanine residues. Inspection of the MS/MS electrospray mass spectrum of the molecular ion of geodiamolide O (**16**) identified an ion at *m/z* 393 Da, attributed to a protonated tyrosine/alanine dipeptide, resulting from cleavage of the C-13/N-12 amide bond and cleavage of the C-8/O bond via a McLafferty rearrangement once again involving H-7. The observation of this fragment indicated that the alanine residue was attached to the amide carbonyl C-11 (δ 168.4) of the methyl iodotyrosine through an amide bond and to the polyketide fragment via an ester linkage. Therefore, the serine residue had to be situated between N-12 of the iodotyrosine residue and C-1 (δ 177.6) of the polyketide moiety.

Geodiamolide P (**17**) gave a molecular ion in the HREIMS consistent with a molecular formula of C$_{28}$H$_{39}$N$_3$O$_7$Br (*m/z* M$^+$ 611.20374/609.20515, ΔM −1.3/−0.3 ppm). The ^{1}H NMR data obtained for **17** (Table 2) were nearly identical with those recorded for geodiamolide O (**16**). The change

in chemical shift of the resonance assigned to H-17 from δ 7.46 in geodiamolide O (**16**) to δ 7.27 in **17**, indicated that **17** is simply the C-18 bromo analogue of **16**. Although no chloro analogue of geodiamolide O (**16**) was found, it is likely to exist as a natural product and, therefore, the name geodiamolide Q has been reserved for the hypothetical structure **18** in anticipation of its future discovery or synthesis. Acid hydrolysis of geodiamolides L (**13**) and O (**16**), followed by derivatization with Marfey's reagent[12] and HPLC analysis, demonstrated that the serine residues in each had the L configuration. Based on the similarity of the ^{1}H and ^{13}C NMR chemical shifts between **13** and **16** and geodiamolide A (**2**),[5] it was assumed that chiral centers in the *N*-methyliodotyrosine, alanine, and polyketide fragments had the same relative configurations in all three molecules.

Geodiamolide R (**19**) exhibited a parent ion in the HREIMS at *m/z* M$^+$ 643.17539, corresponding to a molecular formula of C$_{27}$H$_{38}$O$_7$N$_3$I (ΔM 0.1 ppm). A comparison between the ^{1}H NMR data for **19** and geodiamolide D (**5**) (Table 3) suggested that **19** was simply the serine analogue of geodiamolide D (**5**). The only significant difference in the data is the absence of the alanine methyl doublet, observed at δ 1.25 (Me-26) in the ^{1}H NMR spectrum of geodiamolide D (**5**) and the appearance of a pair of methylene resonances at δ 3.91 (dm, *J* = 8 Hz: H-26) and δ 3.75 (m, H-26′) in ^{1}H NMR spectrum of **19**.

Evidence for the connectivity in **19** was obtained from the ion observed at *m/z* 409 Da in the MS/MS electrospray data. This ion was attributed to the previously observed protonated serine-*N*-methyliodotyrosine dipeptide resulting from the cleavage of the C-13/N-12 amide bond and the

Table 3. ^{1}H NMR Data for Geodiamolides D (**5**)[4] and R (**19**) in CDCl₃ at 500 MHz

position	5 δ_H	19 δ_H
2	2.42 m	2.39 m
3	2.10 m	2.10 m
3′	2.10 m	2.05 m
5	4.99 d (8)	4.97 d (9)
6	2.21 m	2.19 m
7	1.69 m	1.70 m
7′	1.40 m	1.41 m
8	4.86 m	4.88 m
10	4.51 dq (7, 8)	4.53 dq (7, 8)
NH(10)	6.6 d (8)	6.87 d (8)
12	5.08 dd (7, 9)	5.11 dd (7, 9)
14	4.16 dd (1, 18)	4.15 dd (4, 18)
4′	3.77 dd (4, 18)	3.78 dd (3, 18)
NH(14)	6.45 dd (4, 1)	6.47 br s
15	3.25 dd (9, 14)	3.24 dd (9, 14)
15′	2.81 dd (7, 14)	2.81 dd (7, 14)
17	7.51 d (2)	7.50 d (2)
20	6.90 d (8)	6.89 d (8)
21	7.08 dd (2, 8)	7.07 dd (2, 8)
22	1.16 d (7)	1.14 d (7)
23	1.53 d (1)	1.49 s
24	0.90 d (7)	0.89 d (7)
25	1.25d (6)	1.26 d (6)
26	1.30d (7)	3.91 dm (8)
26′		3.75 m
27	2.94 s	2.93 s

C-8/O bond. The similarity between the ^{1}H NMR data of geodiamolide D (**5**) and the ^{1}H NMR data of **19**, combined with the mass spectral evidence for the serine−iodotyrosine partial structure, allowed the structure of **19** to be established. No stereochemical information was obtained for **19**, but it is assumed to have the same relative stereochemistry as all of the other geodiamolides.

There are now **19** known members of the geodiamolide family of cyclic depsipeptides, which have been isolated from sponges in four different genera.[4,5,7,9] Variations have been observed in all three amino acid positions and also in the polyketide portion of the molecule. Amino acid A variants include alanine (**2−7**, **16**, **17**), serine (**13−15**, **19**), β-tyrosine (**9**, **10**), and valine (**20**, **21**); amino acid B variants include only the C-18 halogen atom, with iodine (**2**, **5**, **8**, **9**, **13**, **16**, **19**, **21**), bromine (**3**, **6**, **10**, **11**, **14**, **17**), and chlorine (**4**, **7**, **12**, **15**, **20**) all being observed; amino acid C variants include alanine (**2−4**, **9**, **10**, **13−15**, **20**), glycine (**5−7**, **19**, **21**), and serine (**16**, **17**).

Comparison of the cytotoxicities of the previously reported geodiamolides A−F (**2−7**) and TA (**20**) showed that significant variation in the three amino acid residues causes only minor changes in the levels of cytotoxicity exhibited by the compounds. In contrast, geodiamolide G (**8**) (in vitro human glioblastoma/astrocytoma U373, IC₅₀ 7.7 mg/mL; in vitro human ovarian carcinoma HEY, IC₅₀ 8.6 mg/mL),[5] with its modified polyketide fragment, is significantly less cytotoxic than the analogous geodiamolide A (**2**) (in vitro human glioblastoma/astrocytoma U373, IC₅₀ 0.016 mg/mL; in vitro human ovarian carcinoma HEY, IC₅₀ 0.043 mg/mL).[2] The serine analogues reported in the current study were not available in sufficient quantities to permit determination of accurate in vitro cytotoxicities.

The jaspamide/geodiamolide family of metabolites occurs across a taxonomically distant group of sponge species.[1,4,5,9] To account for this observation, it has been suggested that microorganisms associated with the sponges produce these metabolites.[7] The recent isolation of chondramides, which are jaspamide analogues, from cultures of various strains of *Chondromyces* myxobacteria[14,15] strongly supports the hypothesis of a microbial origin for the jaspamide/geodiamolides.

Experimental Section

General Experimental Procedures. Normal- and reversed-phase TLC were carried out on commercial aluminum-backed Si gel 60 F₂₅₄ (E. Merck, type 5554, 0.25 mm) and glass-backed Whatman MKC₁₈F reversed-phase TLC plates, respectively. TLC plates were eluted with 1:1 EtOAc−hexane, and the spots were visualized by either UV light (254 nm) or a solution of vanillin in a H₂SO₄−EtOH mixture (6% vanillin w/v, 4% H₂SO₄, and 10% H₂O v/v in EtOH). Flash chromatography was performed on 10−40 μ Si gel (Sigma). Gel permeation chromatography was performed using Sephadex LH20 resin. HPLC separations were performed on a Waters 600E HPLC pump/system controller with a Waters 486 tunable absorbance detector. All HPLC solvents were Fischer HPLC grade and were filtered and degassed prior to use. H₂O was purified using a Waters Millipore system. NMR samples were dissolved in CDCl₃ in 5-mm tubes (535 PP), and spectra were recorded at 25 °C with a Bruker AMX 500 spectrometer at 500.13 MHz (^{1}H) or at 125.77 MHz (^{13}C) using a 5-mm inverse-geometry probe. Chemical shifts were referenced to the solvent resonances (CHCl₃ at δ 7.24 ppm, CDCl₃ at δ 77.0 ppm). LREIMS, LCDCIMS, HREIMS, and HRDCIMS were recorded on a Kratos MS50/DS55SM mass spectrometer (70 eV).

Extraction and Isolation. Specimens of *Cymbastela* sp. were collected by hand using scuba on outer reefs at −10 to −25 m near Motupore and Madang in Papua New Guinea. The sponge is arborescent to irregularly flabelliform due to fusion of branches. Branches are often thickened or club-shaped at the end. Color is orange; size, up to 15 cm high; individual branches, 1−1.5 cm in diameter; surface, rough to the touch but otherwise looks smooth. Skeleton is reticulate, with rectangular or polygonal tightly spaced meshes, their sides made up of bundles of 2−10 spicules. Binding spongin is visible at the nodes. From the periphery inward, the skeleton is gradually condensed. Spicules are exclusively oxeas, curved, relatively thin, sharply pointed, size 206−288 × 4−9 = B5m (n = 3D25). The sponge is assigned to the genus *Cymbastela* (family Axinellidae, order Halichondrida) on account of the axinellid arrangement of the skeleton in combination with exclusively short oxeas as spicules. It is an unusual *Cymbastela*, because sponges of this genus are usually cup-shaped, not arborescent. A voucher fragment of the sponge is kept in the collections of the Zoological Museum of Amsterdam under reg.no. 10482.

Freshly collected sponge samples were frozen on-site and transported to Vancouver packed in dry ice. Bioassay-guided fractionation of the *Cymbastela* sp. sponge was performed as follows. Lyophilized sponge (160 g dry wt) was extracted exhaustively with a 1:1 solution of CH₂Cl₂−MeOH (4 × 4 L). The combined organic extracts were filtered and concentrated in vacuo to yield 14 g of a dark yellow/orange oil that was suspended in 250 mL of a 9:1 solution of MeOH−H₂O and extracted with hexanes (3 × 150 mL). The MeOH−H₂O layer was diluted with H₂O to a 4:1 MeOH−H₂O solution and extracted with CCl₄ (3 × 150 mL). Further dilution of the MeOH−H₂O layer with H₂O resulted in a 2:3 MeOH−H₂O solution, which was extracted with CHCl₃ (3 × 150 mL). All traces of MeOH were removed in vacuo, and the resulting aqueous solution was extracted with EtOAc (3 × 150 mL). The cytotoxic activity was concentrated in the CCl₄- and CHCl₃-soluble portions, which were combined and fractionated further by Sephadex LH-20 chromatography, eluting with MeOH, to yield two active fractions, one containing a mixture of geodiamolides and one containing a mixture of hemiasterlin−criamide peptides. Sequential purification of the geodiamolide fraction via Si gel flash column chromatography using a step gradient from 1:1 to 2:1 EtOAc−hexane and reversed-phase HPLC, eluting with 2:3 H₂O−MeOH (Econosil C18 10-μ

column), yielded pure samples of geodiamolides J−P (**11**−**17**) and R (**19**) and the known metabolites, geodiamolides A−G (**2**−**8**).

Geodiamolide J (11): colorless glass (1 mg, 0.0004% dry wt); see Table 1 for ^{1}H NMR data; HRDCIMS (m/z) [M + H]$^+$ calcd for $C_{28}H_{39}N_3O_7{}^{81}Br/C_{28}H_{39}N_3O_7{}^{79}Br$: 610.19509/608.19713; found: 610.19431/608.19508 (ΔM −1.49, −3.38 ppm).

Geodiamolide K (12): colorless glass (0.3 mg, 0.00012% dry wt); see Table 1 for ^{1}H NMR data; HRDCIMS (m/z) [M + H]$^+$ calcd for $C_{28}H_{39}N_3O_7{}^{37}Cl/C_{28}H_{39}N_3O_7{}^{35}Cl$: 565.23688/563.23981; found: 565.23956/563.24011 (ΔM −4.7, −0.5 ppm).

Geodiamolide L (13): colorless glass (2 mg, 0.0008% dry wt); see Table 2 for NMR data; HREIMS (m/z) M$^+$ calcd for $C_{28}H_{40}N_3O_7I$: 658.19892; found: 658.19767 (ΔM −1.91 ppm).

Geodiamolide M (14): colorless glass (1.5 mg, 0.0006% dry wt); see Table 2 for ^{1}H NMR data; HREIMS (m/z) M$^+$ calcd for $C_{28}H_{40}N_3O_7{}^{81}Br/C_{28}H_{40}N_3O_7{}^{79}Br$: 611.20294/609.20496; found: 611.20371/609.20519 (ΔM −1.3, −0.4 ppm).

Geodiamolide N (15): colorless glass (0.8 mg, 0.00032% dry wt); see Table 2 for ^{1}H NMR data, HREIMS (m/z) M$^+$ calcd for $C_{28}H_{40}N_3O_7{}^{37}Cl/C_{28}H_{40}N_3O_7{}^{35}Cl$: 567.25250/565.25549; found: 567.25184/565.25403 (ΔM 1.2, 2.6 ppm).

Geodiamolide O (16): colorless glass (1.5 mg, 0.0006% dry wt); see Table 2 for NMR data; HREIMS (m/z) M$^+$ calcd for $C_{28}H_{40}N_3O_7I$: 658.19892; found: 658.19880 (ΔM −0.19 ppm).

Geodiamolide P (17): colorless glass (1.5 mg, 0.0006% dry wt); see Table 2 for ^{1}H NMR data; HREIMS (m/z) M$^+$ calcd for $C_{28}H_{40}N_3O_7{}^{81}Br/C_{28}H_{40}N_3O_7{}^{79}Br$: 611.20294/609.20496; found: 611.20374/609.20515 (ΔM −1.3/−0.3 ppm).

Geodiamolide R (19): colorless glass (1.0 mg, 0.0004% dry wt); see Table 3 for ^{1}H NMR data; HREIMS (m/z) M$^+$ calcd for $C_{27}H_{38}N_3O_7I$: 643.17548; found: 643.17539 (ΔM 0.1 ppm).

Acid Hydrolysis of Geodiamolides. Pure individual geodiamolides (ca. 0.25 mg each) were dissolved in 1 mL of freshly distilled constant boiling HCl, and the resulting solution was heated at 108 °C with stirring for 16 h in a threaded Pyrex tube sealed with a Teflon screw cap. The cooled reaction mixture was evaporated to dryness, and traces of HCl were removed from the residual hydrolyzate by repeated evaporation from H_2O (3 × 3 mL).

Marfey's Reagent Amino Acid Analysis.[13] To a 1-mL vial containing 2 mmol of pure amino acid standard in 80 mL of H_2O was added 2.8 mmol of N-α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) in 170 mL of Me_2CO followed by 20 mL of 1 N NaHCO3. The mixture was heated for 1 h at 40 °C. After cooling to room temperature, 10 mL of 2 N HCl were added, and the resulting solution was filtered through a 4.5-mm filter and stored in the dark until HPLC analysis. To prepare FDAA derivatives of the amino acids in the hydrolyzate of the geodiamolides, a 90-mL aliquot containing 0.9 mg of amino acid mixture was reacted with 2.86 mmol of FDAA in 172 mL of Me_2CO as described above. A 10-mL aliquot of the resulting mixture of FDAA derivatives was analyzed by reversed-phase HPLC. A linear gradient of (A) 9:1 triethyl-ammonium phosphate (50 mM, pH 3.0)/MeCN and (B) MeCN, with 0% B at the start to 40% B over 60 min (flow rate 1 mL/min) was used to separate the FDAA derivatives, which were detected by UV at 340 nm. Each peak in the chromatographic trace was identified by comparing its retention time with that of the FDAA derivative of the pure amino acid standard and by co-injection. In all cases a peak was observed at ca. 35.7 min, which was attributed to excess FDAA. The HPLC retention times are tabulated below:

HPLC Retention Times for Marfey's Derivatives of D- and L-Serine (in Minutes)

	L-ser	D/L-ser	FDAA
standards	26.0	L 25.9	35.8
		D 26.6	
geodiamolide L (**13**)	25.9		35.7
geodiamolide O (**16**)	25.9		35.8

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