Batzelladines F−I, Novel Alkaloids from the Sponge *Batzella* sp.: Inducers of p56^{lck}-CD4 Dissociation

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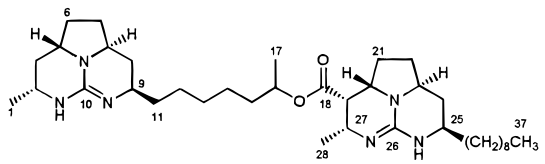
The sponge *Batzella* sp. from Jamaica contains four new alkaloids, batzelladines F−I (**1**-**4**), that induce the p56^{lck}−CD4 dissociation. Batzelladines F−I were isolated using a bioassay-directed fractionation scheme and were identified by interpretation of spectroscopic data. Batzelladines F, G, and H + I were active in the p56^{lck}−CD4 dissociation assay at micromolar concentrations.

Introduction

It has been established that immunological responses by T lymphocytes are mediated by CD4 expressed on cell surfaces.[1−3] An important step toward understanding the mode of action of CD4 was taken when it was found that its cytoplasmic tail interacted with a protein tyrosine kinase called p56^{lck}. In fact, it has been reported that the association of p56^{lck} with CD4 is required for antigenic activation to take place.[4] This allowed a screening strategy to be implemented for finding selective agents of immunosuppression which could then be optimized for the treatment of autoimmune diseases (murine lupus, allergic encephalomyelitis, and adjuvant-induced arthritis) and allograft rejection.

Results and Discussion

Marine organisms are a very productive source of compounds with interesting biological activities.[5] As part of our continuing search for biologically active natural products for the development of immunosuppressive agents, we initiated a high throughput screen to evaluate the ability of natural product extracts to induce the p56^{lck}−CD4 dissociation. Over 3500 extracts were screened. One of the two extracts which showed activity was found to contain a mixture of halitoxins,[6] which are regarded as nuisance compounds because they have demonstrated a very broad range of nonspecific activity across many types of target, and that extract was not pursued. The other extract was the methanol extract of a sponge *Batzella* sp., collected in Jamaica, which showed consistent activity in p56^{lck}−CD4 bioassays and was selected for fractionation. We had earlier reported the isolation and identification of five interesting polycyclic guanidine alkaloids, batzelladines A−E, from a sponge of the genus *Batzella*, collected in the Bahamas.[7] These compounds were the first natural products to inhibit the binding of HIV-1 gp120 to human CD-4. We now report the isolation and identification of four novel guanidine alkaloids, batzelladines F−I (**1**−**4**) which show remarkable activity in the p56^{lck}−CD4 dissociation assay.



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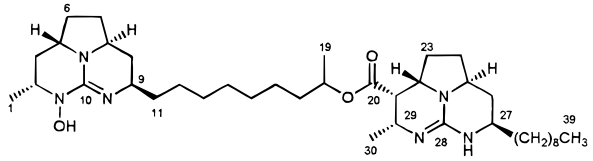

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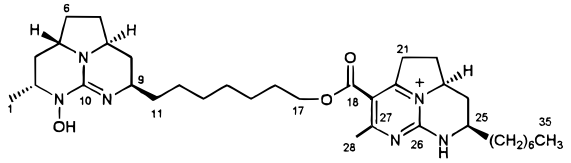

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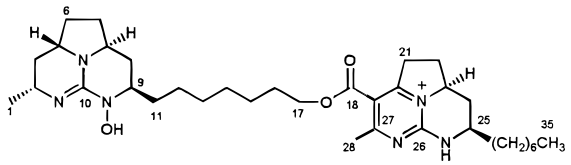

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The freeze-dried sponge was extracted sequentially with ethyl acetate and methanol. Bioassay-guided fractionation of the 1-butanol soluble portion of the methanol extract, which showed ability to induce p56^{lck}−CD4 dissociation, using Sephadex LH-20 followed by silica gel column chromatography, afforded several active fractions. Further purification of the active fractions by silica gel PTLC and RP-18 HPLC, including preparative LC-

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Table 1. ^{1}H NMR Data [δ (mult, no. of H, J in hertz)] for batzelladines F−I (1−4) in CD_3OD

no.	1	2	3	4
1	1.26 (d, 3 H, 6.3)	1.32 (d, 3 H, 6.3)	1.33 (d, 3 H, 6.3)	1.33 (d, 3H, 6.3)
2	3.52 (m, 1 H)	3.76 (m, 1 H)	3.77 (m, 1 H)	3.57 (m, 1 H)
3	2.20 (m, 1 H)	2.32 (m, 1 H)	2.31 (m, 1 H)	2.29 (m, 1 H)
	1.25 (m, 1 H)	1.61 (m, 1 H)	1.60 (m, 1 H)	1.28 (m, 1 H)
4	3.72 (m, 1 H)	3.76 (m, 1 H)	3.77 (m, 1 H)	3.77 (m, 1 H)
5	2.20 (m, 1 H)	2.21 (m, 1 H)	2.20 (m, 1 H)	2.20 (m, 1 H)
	1.67 (m, 1 H)	1.65 (m, 1 H)	1.63 (m, 1 H)	1.63 (m, 1 H)
6	2.20 (m, 1 H)	2.21 (m, 1 H)	2.20 (m, 1 H)	2.20 (m, 1 H)
	1.67 (m, 1 H)	1.65 (m, 1 H)	1.63 (m, 1 H)	1.63 (m, 1 H)
7	3.72 (m, 1 H)	3.72 (m, 1 H)	3.72 (m, 1 H)	3.72 (m, 1 H)
8	2.28 (m, 1 H)	2.34 (m, 1 H)	2.35 (m, 1 H)	2.35 (m, 1 H)
	1.21 (m, 1 H)	1.27 (m, 1 H)	1.27 (m, 1 H)	1.55 (m, 1 H)
9	3.40 (m, 1 H)	3.46 (m, 1 H)	3.47 (m, 1 H)	3.65 (m, 1 H)
11	1.60 (m, 1 H)	1.83 (m, 1 H)	1.82 (m, 1 H)	1.85 (m, 1 H)
	1.54 (m, 1 H)	1.55 (m, 1 H)	1.55 (m, 1 H)	1.65 (m, 1 H)
12	1.30 (m, 2 H)	1.30 (m, 2 H)	1.40 (m, 2 H)	1.38 (m, 2 H)
13	1.30 (m, 2 H)	1.30 (m, 2 H)	1.38 (m, 2 H)	1.38 (m, 2 H)
14	1.30 (m, 2 H)	1.30 (m, 2 H)	1.38 (m, 2 H)	1.38 (m, 2 H)
15	1.60 (m, 1 H)	1.30 (m, 2 H)	1.42 (m, 2 H)	1.42 (m, 2 H)
	1.54 (m, 1 H)			
16	4.97 (m, 1 H)	1.30 (m, 2 H)	1.78 (m, 2 H)	1.78 (m, 2 H)
17	1.24 (d, 3 H, 6.5)	1.64 (m, 1 H)	4.33 (t, 2 H, 6.7)	4.33 (t, 3 H, 6.7)
		1.57 (m, 1 H)		
18		4.97 (m, 1 H)		
19	3.06 (dd, 1 H, 4.6, 3.3)	1.23 (d, 3 H, 6.3)		
20	3.94 (m, 1 H)			
21	2.24 (m, 1 H)	3.05 (dd, 1 H, 4.8, 3.3)	3.61 (dd, 1 H, 19.3, 8)	3.61 (dd, 1 H, 19.3, 8)
	1.62 (m, 1 H)		3.43 (dd, 1 H, 19.3, 8)	3.43 (dd, 1 H, 19.3, 8)
22	2.18 (m, 1 H)	3.93 (m, 1 H)	2.65 (m, 1 H)	2.65 (m, 1 H)
	1.63 (m, 1 H)		2.01 (m, 1 H)	2.01 (m, 1 H)
23	3.52 (m, 1 H)	2.24 (m, 1 H)	4.57 (m, 1 H)	4.57 (m, 1 H)
		1.62 (m, 1 H)		
24	2.34 (m, 1 H)	2.18 (m, 1 H)	2.57 (dt, 1 H, 13.5, 4)	2.57 (dt, 1 H, 13.5, 4)
	1.42 (m, 1 H)	1.63 (m, 1 H)	1.58 (dt, 1 H, 13.5, 11.4)	1.58 (dt, 1 H, 13.5, 11.4)
25	3.53 (m, 1 H)	3.52 (m, 1 H)	3.81 (m, 1 H)	3.81 (m, 1 H)
26		2.34 (m, 1 H)		
		1.42 (m, 1 H)		
27	3.82 (m, 1 H)	3.52 (m, 1 H)		
28	1.27 (d, 3 H, 6.7)		2.74 (s, 3 H)	2.74 (s, 3 H)
29	1.64 (m, 1 H)	3.82 (m, 1 H)	1.85 (m, 1 H)	1.85 (m, 1 H)
	1.54 (m, 1 H)		1.60 (m, 1 H)	1.60 (m, 1 H)
30	1.33 (m, 2 H)	1.27 (d, 3 H, 6.7)	1.47 (m, 2 H)	1.47 (m, 2 H)
31	1.30 (m, 2 H)	1.64 (m, 1 H)	1.40 (m, 2 H)	1.40 (m, 2 H)
		1.57 (m, 1 H)		
32	1.30 (m, 2 H)	1.33 (m, 2 H)	1.38 (m, 2 H)	1.38 (m, 2 H)
33	1.30 (m, 2 H)	1.30 (m, 2 H)	1.38 (m, 2 H)	1.38 (m, 2 H)
34	1.30 (m, 2 H)	1.30 (m, 2 H)	1.38 (m, 2 H)	1.38 (m, 2 H)
35	1.30 (m, 2 H)	1.30 (m, 2 H)	0.90 (t, 3 H, 7)	0.90 (t, 3 H, 7)
36	1.30 (m, 2 H)	1.30 (m, 2 H)		
37	0.90 (t, 3 H, 7)	1.30 (m, 2 H)		
38		1.30 (m, 2 H)		
39		0.89 (t, 3 H, 7)		

MS, yielded ptilomycalin A,[8] formiamycalin,[9] crambescidin 800,[10−12] and the four novel alkaloids, batzelladines F (**1**, 0.018% dry weight), G (**2**, 0.024% dry weight), and H + I (**3** + **4**, 0.009% dry weight), which were isolated as formate salts.

Batzelladine F (**1**) was isolated as a colorless gum, $[\alpha]_D = +19.4°$, and displayed a molecular ion at m/z 624 (two exchangeable protons) in the DCI mass spectrum which corresponded to a molecular formula of $C_{37}H_{64}N_6O_2$ (HRFABMS) that required nine degrees of unsaturation. The FT-IR microscopy spectrum of batzelladine F (**1**) contained bands at 3600−3100 (N−H), 1724 (C=O), 1644 and 1622 (C=N), and 1326 cm^{-1} (C−N). These bands were similar to those of batzelladine A (**5**), the stereochemistry of which has recently been revised,[13] and related compounds isolated previously. From the IR and ^{1}H NMR spectra, it was clear that batzelladine F (**1**) belonged to the batzelladine family of compounds. The ^{1}H NMR spectrum of batzelladine F (**1**) included an oxygenated methine multiplet at δ 4.97 and nine overlapping methine multiplets in the region between δ 3.94 and 3.06 (Table 1). The upfield portion of the proton spectrum contained several methylene multiplets between δ 2.34 and 1.21 and four methyl resonances. Three of the methyl signals were doublets at δ 1.27, 1.26, and 1.24, and the remaining methyl signal was a triplet at 0.90.

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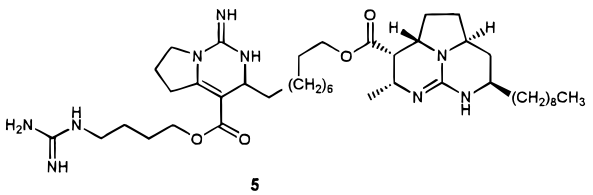
Table 2. ^{13}C NMR Data (CD_3OD) for Batzelladines F−I (1−4)

no.	1	2	3	4
1	20.7	18.6	18.4	20.3
2	47.2	55.6	55.8	47.8
3	36.9	37.3	37.3	36.4
4	57.5	56.0	56.1	55.7
5	31.1	30.9	30.9	30.9
6	31.1	31.6	31.2	31.2
7	57.4	58.6	58.6	58.4
8	34.8	33.8	33.7	34.7
9	51.6	52.0	52.0	59.7
10	151.2	152.8	152.8	152.9
11	35.8	35.0	35.0	35.1
12	26.2	26.1	26.0	25.8
13	30.5	30.5	30.5	30.5
14	26.5	30.5	30.5	30.5
15	36.9	30.5	27.1	27.1
16	73.3	26.5	29.7	29.7
17	20.5	36.9	67.0	67.0
18	170.3	73.2	164.7	164.7
19	45.6	20.5	112.8	112.8
20	57.9	170.3	166.9	166.9
21	29.2	45.6	34.3	34.3
22	31.4	57.9	30.3	30.3
23	57.3	29.3	63.1	63.1
24	34.2	31.5	31.0	31.0
25	53.2	57.3	53.3	53.3
26	151.6	34.2	152.2	152.2
27	49.9	53.1	178.2	178.2
28	18.5	151.7	26.4	26.4
29	36.9	49.8	35.1	35.1
30	26.2	18.5	26.0	26.0
31	30.5	36.9	30.5	30.5
32	30.5	26.2	30.5	30.5
33	30.5	30.5	32.9	32.9
34	30.5	30.5	23.7	23.7
35	33.0	30.5	14.4	14.4
36	23.7	30.5		
37	14.5	33.1		
38		23.7		
39		14.5		

The ^{13}C GASPE NMR spectrum of **1** revealed four downfield quaternary resonances, an ester carbonyl at δ 170.3, the formate anion (169.9) and two guanidine carbons at 151.6 and 151.2 (Table 2). There was also an oxygenated methine signal at δ 73.3 and nine additional methine signals between 57.9 and 45.6. In the upfield portion of the spectrum there were twenty methylene resonances between δ 36.9 and 23.7 and four methyl signals between 20.7 and 14.5. The two guanidine and nine non-oxygen-bearing methine carbons in the downfield region indicated that there were two tricyclic guanidinium units present in **1** connected by an aliphatic chain. The tricyclic guanidinium ring system present in batzelladines A−E has been well characterized by both ^1H and ^{13}C NMR studies.[7,13]

There was a subset of the ^1H and ^{13}C NMR signals which occurred at the same chemical shifts as those of the tricyclic guanidinium moiety in batzelladine A (**5**), and a second subset of signals which were similar but not identical to the first subset. Assignment of the



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carbons in the right-hand tricyclic guanidinium unit, which was identical to that of batzelladine A (**5**), was based on the following observations. The COSY spectrum revealed two contiguous chains from Me-28 to CH_2-21 and from CH_2-22 to CH-25, which is coupled to a methylene chain that terminates at Me-37. The ^1H and ^{13}C NMR chemical shifts of the Me-28 to Me-37 signals confirmed that the right-hand tricyclic guanidinium unit in batzelladine F (**1**) was identical to its counterpart in batzelladine A (**5**). The length of the C_9 aliphatic side chain attached at C-25 was established by interpretation of mass spectral fragmentation data.

In the HMBC experiment, the CH_3-28 protons showed correlations to C-27 (δ 49.9) and C-19 (45.6), and the H-19 signal correlated to C-27, C-20 (57.9), and C-18 (170.3). Thus the ester function was attached to the tricyclic guanidinium ring system at C-19. In similar fashion, H-20 was correlated to C-27 and C-21 (δ 29.2) and on the opposite side of the same tricyclic guanidinium unit both H-23 and H-25 correlated to C-24 (34.2), while H-25 showed additional interactions with C-29 (36.9) and C-30 (26.2) on the aliphatic side chain. The CH_3-37 signal showed correlations to C-35 (δ 33.0) and C-36 (23.7) at the terminus of the side chain.

The second subset of signals, representing the left-hand tricyclic guanidinium portion of batzelladine F (**1**), was similar but not identical to the right-hand subset. The COSY experiment indicated two contiguous chains from CH_3-1 to CH_2-5 and from CH_2-6 to CH-9. The major difference between the two tricyclic ring systems is that there is a methylene at C-3 in the left-hand ring system and a methine at C-19 in the right-hand ring system. The H-9 signal was coupled to a second aliphatic chain that terminated with a methyl doublet at δ 1.24 (CH_3-17) that was coupled to a methine signal at 4.97 (CH-16). Thus, the right-hand portion of the molecule was connected at C-16 through an ester linkage.

Assignments for the carbons in the left-hand tricyclic guanidinium moiety were made in a fashion analogous to that used for the right-hand side and were based on the chemical shift and correlation data. In the HMBC experiment, the CH_3-1 signal at δ 1.26 was correlated to C-2 (47.2) and C-3 (36.9), the H-3 signal was correlated to C-4 (57.5), and the H-6 signals were correlated to C-5 (57.4) and C-7 (51.6). The H-11 protons at δ 1.60 and 1.54 were also correlated to C-7. The CH_3-17 signal at δ 1.24 showed correlations to C-16 (δ 73.3) and C-15 (36.9). The chemical shift of C-16 suggested an ester linkage at that point. The C-18 ester carbonyl showed correlations to H-16 as well as to H-19, H-20, and H-27 in the right-hand tricyclic guanidinium moiety. Thus **1** consisted of two nearly identical tricyclic guanidinium ring systems connected through an ester linkage. The mass spectral fragmentation patterns required five methylene groups in the connecting chain.

There were no HMBC correlations to help differentiate between the C-10 and C-26 guanidine signals at δ 151.6 and 151.2. Therefore, the former was assigned to C-26 in the right-hand portion of **1** by analogy to the δ 151.6 chemical shift of its counterpart (C-31) in batzelladine A (**5**). By default, the latter signal was assigned to C-10. The final ^1H and ^{13}C NMR assignments are presented in Tables 1 and 2, respectively.

The relative stereochemistry of both tricyclic ring systems in **1** was determined to be the same as that found in batzelladine A (**5**).[7,13] As in batzelladine A (**5**), the H-19 signal appeared as a doublet of doublets (J = 3.3 and 4.6 Hz), implying a *syn* relationship to H-20 and H-27, and the presence of large couplings indicated that

H-24$_{ax}$ was trans-periplanar to H-23 and H-25. There was also an NOE enhancement of H-21α (δ 1.62) on irradiation of H-23, proving that H-23 and H-25 were situated on the opposite face of the tricyclic ring system from H-19, H-20, and H-27. Analogous results were observed for the left-hand tricyclic guanidinium moiety. The stereochemical relationship between the two tricyclic guanidinium units could not be determined nor could the relative stereochemistry at C-16. The absolute stereochemistry was not determined.

Batzelladine G (**2**) was isolated as a colorless powder and had a molecular ion at *m/z* 668 in its DCIMS. The molecular formula was determined to be $C_{39}H_{68}N_6O_3$ with two exchangeable hydrogens, and this formula again indicated the presence of nine double bond equivalents. The FT-IR microscopy spectrum of **2** was similar to that observed for **1**, and it was clear that the two compounds were very similar. The molecular formula of batzelladine G (**2**) differs from that of batzelladine F (**1**) by the addition of two methylene groups and an oxygen atom. Analysis of the ^{13}C GASPE NMR spectrum of **2** revealed the addition of two methylene carbon signals in the region associated with the alkyl chains, but there was no evidence of an additional carbon bearing oxygen. It was therefore concluded that the additional oxygen atom was attached to one of the guanidinium nitrogens.

The ^{1}H and ^{13}C NMR signals assigned to the right-hand tricyclic ring system were almost identical to the corresponding signals for batzelladine F (**1**). However, hetero- and homonuclear correlation data indicated that the C-2 signal in the left-hand tricyclic ring system in **2** was located more than 8 ppm downfield from the corresponding signal in **1**. In the HMBC experiment, the CH_3-1 signal at δ 1.32 was correlated with C-2 (55.6) and C-3 (37.3), whereas in **1** the C-2 signal occurred at 47.2. The guanidinium carbon, C-10, also moved 1.6 ppm further downfield in **2**. Thus the *N*-hydroxyl group in batzelladine G (**2**) must be positioned between C-2 and C-10. Further support for this assignment came from analysis of a ^{1}H NMR spectrum recorded in DMSO-d_6, in which the exchangeable protons were observed. The *N*-hydroxyl proton signal was observed as a sharp singlet at δ 10.51. Saturation of this singlet produced a weak but significant NOE enhancement of the CH_3-1 signal, which confirmed the location of the *N*-hydroxyl group. The ^{1}H and ^{13}C NMR assignments are presented in Tables 1 and 2, respectively.

The mass spectral fragmentation pattern was used to establish that the two additional methylene groups present in **2** were situated in the central chain connecting the left-hand tricyclic ring to the ester oxygen. This chain was five methylene units in length in **1** and 7 methylene units long in **2**. Fragments arising from the left-hand tricyclic ring system were observed to be 16 Da higher in **2** than in **1** because of the presence of the *N*-hydroxyl group.

By analysis of coupling constant and NOE difference data, it was demonstrated that the stereochemistry about each of the tricyclic ring systems in batzelladine G (**2**) was the same as in batzelladine F (**1**). Again, no further stereochemical details could be determined.

Batzelladines H (**3**) and I (**4**) were isolated as a 43:57 mixture of isomers that had a molecular weight of 609 Da. The molecular formula was determined by high resolution mass spectrometry to be $C_{35}H_{57}N_6O_3$, with two exchangeable hydrogens. The FT-IR microscopy spectrum of individual crystals of **3** and **4** showed that they were formate salts with infrared bands that were similar to those observed for **1** and **2**. The UV spectrum contained a band at 258 nm, which suggested the presence of a heteroaromatic moiety. Although the spectral characteristics showed similarities with those of **1** and **2**, the molecular formula indicated that both **3** and **4** were formate salts of cationic molecules, which may explain why they proved inseparable. The approximately 3:2 ratio of compounds allowed the signals in the ^{1}H and ^{13}C NMR spectra to be separated into two sets. Furthermore, the HMQC and HMBC experiments allowed a complete assignment of the ^{1}H and ^{13}C NMR signals as shown in Tables 1 and 2, respectively.

The ^{1}H NMR spectrum of **3** and **4** included six overlapping methine multiplets between δ 4.6 and 3.4 instead of the nine observed for **1** and **2**. The methine signal at δ 4.57 was noticeably downfield compared with corresponding signals in **1** and **2**. Furthermore, only three methyl signals were observed at δ 2.74 (s, 3H), 1.33 (d, 3 H), and 0.90 (t, 3 H), at identical chemical shifts for both compounds.

The ^{13}C GASPE NMR spectrum of **3** and **4** showed six quaternary signals between δ 150 and 180, with only one of those signals being doubled, indicating that **3** and **4** contained two more double bonds than did **1** or **2**. There was an oxymethylene signal at δ 67.0, a complex group of methine signals between 47 and 65, which accounted for six methine carbons in each compound, and three methyl signals at 26.4, 18.4, and 14.4 for **3** and 26.4, 20.3, and 14.4 for **4**. The missing methyl group was that on the aliphatic chain joining the two tricylic portions of **1** and **2**, which was replaced by an unsubstituted aliphatic chain in **3** and **4**. The additional downfield signals suggested that one of the nitrogen-containing rings was aromatic, accounting for the difference of three methine signals.

In batzelladine H (**3**), the ^{1}H and ^{13}C signals corresponding to the left-hand tricyclic ring system were nearly identical to those of **2** suggesting that the *N*-hydroxyl group was again situated on the nitrogen between C-2 and C-10 in **3**. The signals due to C-2 and C-10 occurred at δ 55.8 and 152.8 in both **2** and **3**. Hetero- and homonuclear correlation data indicated that the C-19, -20, and -27 signals were at δ 112.8, 166.9, and 178.2 and thus indicated the position of the aromatic ring in **3**. Specifically, the CH_3-28 methyl singlet at δ 2.74, which is at unexpectedly low field even for a methyl on an aromatic ring, correlated with C-27 and C-19 while the H-21 signals at 3.61 and 3.43 showed a correlation to C-20. The ^{1}H chemical shifts of the H-21 and H-23 signals were more than the 1 ppm downfield of the corresponding signals in **1** or **2**, confirming that they were adjacent to an aromatic ring. Furthermore, a 6 ppm upfield shift of the C-18 signal to δ 164.7 was observed due to the ester being conjugated to the aromatic ring.

The mass spectral fragmentation pattern was used to establish that the central chain connecting the two ring systems was seven carbons long and terminated with a primary ester. The HMBC correlation between H-17 (δ 4.33) and the ester carbonyl confirmed this proposal. Fragments arising from the right-hand ring system established the presence of a seven-carbon chain, unlike the C_9 chains observed in **1** and **2**.

From the coupling constants and NOE difference data, it was demonstrated that the left-hand tricyclic guanidinium moiety in **3** retained the same relative stereochemistry as in **1** and **2**. In the right-hand portion of **3**,

H-24_{ax} at δ 1.58 was transperiplanar to H-23 at 4.57 and H-25 at 3.81. Neither the stereochemical relationship between the two tricyclic guanidinium units nor the absolute configuration could be determined.

The ^{1}H and ^{13}C signals assigned to batzelladine I (**4**), which is an isomer of batzelladine H (**3**), indicated that the right-hand ring system was identical in both compounds. There were some differences, however, between the signals assigned to the left-hand ring system. Interpretation of the HMQC and HMBC experiments indicated a 7.7 ppm downfield shift for C-9 and an 8.0 ppm upfield shift for C-2 in going from **3** to **4**. In particular, the CH_3-1 signal at δ 1.33 showed a correlation to C-2 (47.8) and C-3 (36.4), and H-7 was directly correlated to C-9 (59.7). The C-10 guanidine carbon did not change appreciably. These changes indicated that the *N*-hydroxy group was located between C-9 and C-10 in **4**. All of the other signals retained nearly identical chemical shifts and correlations.

Mass spectral fragmentation was again used to establish that the lengths of aliphatic chains were identical in compounds **3** and **4**. The coupling constants and NOE difference data demonstrated that the left- and right-hand tricyclic ring systems in **4** retained the same relative stereochemistry as their counterparts in **3**. Again, no further stereochemical information could be obtained.

At dose ranges higher than 30 μg/mL, the p56^{lck}−CD4 active fractions produced an apparent induced dissociation greater than 100%. We attributed this as being the result of assay interference unrelated to p56^{lck}−CD4 association. When these fractions were further purified into three compounds, batzelladine F (**1**) was clearly responsible for the observed assay perturbation. In contrast, batzelladines G (**2**) and H + I (**3** + **4**) demonstrated an ability to induce dissociation of the p56^{lck}−CD4 complex without interfering with the assay at a concentration of 24 and 7.1 μM, respectively. The high degree of specificity of the compounds found in this screen is illustrated by the fact that of 40000 retrospective file compounds screened (not connected with the batzelladine project), only two compounds with activities comparable to batzelladines F (**1**) and G (**2**) were encountered. Although batzelladines F−I (**1**−**4**) exhibited only moderate potency, such compounds provide the basis for rational design of analogs possessing increased biochemical activity.

Experimental Section

FT-IR Transmittance Spectra. FT-IR microscopy transmittance spectra were measured using a NICPLAN FT-IR microscope equipped with a liquid nitrogen-cooled MCT-A (mercury−cadmium−telluride) detector. Each microscope sample was prepared as thin section on a FT-IR compression cell equipped with diamond windows. These flattened samples were placed under a nitrogen purge prior to and during analysis to facilitate water removal. Spectra from all samples resulted from signal averaging of 300 scans obtained at a spectral resolution of 4 cm^{-1}. All of the results obtained were then recorded as transmittance spectra so that comparison with a previous reference compound was possible.

When observed under the microscope, the mixture of batzelladines H (**3**) and I (**4**) showed two distinct crystal types. Small (<10 μm) needle-shaped crystals of batzelladine H (**3**) were easily isolated, and a reference FT-IR spectrum was first obtained for these. The second type of crystals were much larger (20−50 μm) and platelike, but their surfaces were coated with the needles of batzelladine H. The FT-IR spectrum of batzelladine I (**4**) was obtained by difference IR spectroscopy where the spectrum of the needles was subtracted from the spectrum of the coated plates.

Biological Assay. High protein binding plates were coated overnight with 1F3 antibody (250 ng/well) in 0.1 M sodium bicarbonate, pH 9.2. The coating buffer was decanted and the wells washed twice with PBS prior to blocking for 30 min at 37 °C with PBT buffer (PBS containing 0.5% BSA (w/w) and 0.05% Tween 20 (v/v)). On the day of the assay, CEM cells were counted with trypan blue on a hemacytometer to ensure a cell density of ca. 1×10^6. The cells were spun down at 800*g* (1200 rpm) for 10 min and washed twice with resuspension in PBS buffer. All further manipulations of the cells and resulting extract were done on ice. Cells were disrupted by occasional vortexing for 30 min with an extraction buffer (50 mM Tris, 2 mM EDTA, 0.15 M NaCl, 3% NP-40 (w/v), 50 mM NaF, 0.1 mM $NaVO_3$) at a cell density of 100×10^6/mL. On the day of the experiment, 48 mL of the extraction buffer was supplemented with 50 μL of 0.1 M PMSF in DMSO, 250 μL of a 2 mg/mL solution of aprotinin, 0.5 mg TLCK, and 0.5 mg of leupeptin. The mixture was then spun at 12000*g* (15000 rpm) for 20 min at 4 °C. The supernatant was removed and diluted 1:16 with PBT. All of the remaining ELISA steps were performed by an Orca robotic arm which enabled high throughput screening to be done overnight. Blocking buffer was decanted from the assay plates immediately prior to running the assay. Inhibitor stock solutions (10 μL in 10% DMSO) were added to diluted cell extract (90 μL) and left for 3 h at room temperature. The wells were washed with PBS and treated with a 1:300 dilution of anti-human lck kinase (Upstate Biotechnology Incorporated) for 1 h at 4 °C. After washing the wells again, goat-anti-rabbit horse raddish peroxidase (8 μL stock into 50 mL of PBT) was added and incubated for 30 min at 4 °C. After a final plate washing, 100 μL OPD substrate (Sigma) was added in substrate buffer (0.1 M citric acid added to 0.1 M dibasic sodium phosphate to pH 5.0) and allowed to turn over for 20 min at room temperature. Reaction of the substrate was quenched with 50 μL of 4.5 M sulfuric acid. The plates were then read in a plate reader with the wavelength set at 490 nm.

Collection, Extraction, and Isolation. The sponge *Batzella* sp. was collected by hand using SCUBA at a depth of 15 m at Discovery Bay, Jamaica, in December, 1992. Specimens were immediately frozen and kept at −20 °C until extraction. The freeze-dried sponge (120 g) was extracted with EtOAC and MeOH to give 3.5 and 10.9 g of extracts, respectively. The MeOH extract, which exhibited p56^{lck}−CD4 activity, was dissolved in H_2O (100 mL) and extracted with *n*-BuOH (3 × 100 mL). The active *n*-BuOH soluble portion, after removal of the solvent, yielded a residue (2.95 g) which was chromatographed on a column of Sephadex LH-20 using MeOH as eluant. The p56^{lck}−CD4 active fractions were monitored by bioassay and pooled. Combined active fractions (0.92 g), which were mixtures of several compounds, were subjected to a silica gel column chromatography (Kieselgel-60, 230−400 mesh), eluting with a solvent system MeOH:CH_2Cl_2:H_2O:HCOOH (15:85:1.5:2.5). Several fractions (15 mL each) were collected and monitored by TLC. Identical fractions were combined to afford ten (A−J) individual fractions. Fractions D (75 mg), E (67 mg), and F (53 mg), after extensive silica gel PTLC using a MeOH:CH_2Cl_2:H_2O:HCOOH (25:75:2.5:3.5) solvent system, yielded ptilomycalin A (56 mg, 0.047% dry weight), formiamycalin (41 mg, 0.034% dry weight), and crambescidin 800 (24 mg, 0.02% dry weight). Fraction A (50 mg), after silica gel PTLC employing MeOH:EtOAC:H_2O:HCOOH (40:60:1.5:2.5) followed by RP-18 HPLC using H_2O:CH_3CN:0.1 TFA (gradient) as eluant, yielded batzelladine F (**1**, 22 mg, 0.018% dry weight). Fraction B (59 mg) was purified first by silica gel PTLC MeOH:CH_2Cl_2:H_2O:HCOOH (25:75:1.5:2.5) and finally by RP-18 HPLC H_2O:CH_3CN:0.1 TFA (gradient) to obtain batzelladine G (**2**, 29 mg, 0.024% dry weight). Fraction C (47 mg) appeared to be homogeneous by TLC, but was found to be a mixture of several compounds by HPLC. One of the major components of the mixture was purified by preparative LC-MS using H_2O:CH_3CN:0.1 TFA (gradient) solvent system to afford batzella-

dine H and I (**3** and **4**, 11 mg, 0.009% dry weight) as a 43:57 mixture determined by ^{1}H NMR spectroscopy.

Batzelladine F (**1**): colorless gum, $[\alpha]_D = +19.4°$ ($c = 0.87$, MeOH); UV (MeOH) λ_{max} 236, 254, 296 nm; IR (neat) ν_{max} 3600−3100, 3000−2800, 1724, 1644, 1622, 1450, 1326, 1182, 705 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS m/z 624 $(M)^+$; HRFABMS calcd for $C_{37}H_{64}N_6O_2$ m/z 624.5090, found m/z 624.5096.

Batzelladine G (**2**): colorless powder, $[\alpha]_D = +14.7°$ ($c = 0.79$, MeOH); UV (MeOH) λ_{max} 233, 258, 295 nm; IR (neat) ν_{max} 3600−3100, 3000−2800, 1724, 1637, 1624, 1378, 1324, 1181, 1123, 1028, 703 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS m/z 668 $(M)^+$; HRFABMS calcd for $C_{39}H_{68}N_6O_3$ m/z 668.5353, found m/z 668.5342.

Batzelladine H + I (**3** + **4**): colorless oil, $[\alpha]_D = +33.7°$ ($c = 0.56$, MeOH); UV (MeOH) λ_{max} 217, 258 nm; IR (neat) ν_{max} for **batzelladine H**, 3342, 3288, 3276, 2924, 2856, 1720, 1639, 1596, 1431, 1380−1200,1162, 1119, 1059, 1032, cm^{-1}; IR (neat) ν_{max} for **batzelladine I**, 3219, 3253, 3136, 2926, 2854, 1720, 1639, 1596, 1447, 1433, 1380−1200, 1161, 1122 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS m/z 609 $(M)^+$; HRFABMS calcd for $C_{35}H_{57}N_6O_3$ m/z 609.4492, found m/z 609.4488.

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Supporting Information Available: Copies of the ^{1}H NMR, ^{13}C GASPE NMR, COSY, HMQC, HMBC, and mass spectral data for batzelladines F and G and the ^{1}H NMR, ^{13}C GASPE NMR, COSY, HMQC, and HMBC data for a mixture of batzelladines H and I (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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