The Lobatamides, Novel Cytotoxic Macrolides from Southwestern Pacific Tunicates †

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The Lobatamides, Novel Cytotoxic Macrolides from Southwestern Pacific Tunicates†

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Novel macrolides, lobatamides A−F (**1**−**6**), have been isolated from shallow water Australian collections of *Aplidium lobatum*, from a deep water collection of *Aplidium* sp., and from an unidentified Philippine ascidian. Full details of the isolation and structure elucidation of **1**−**6** are provided herein, along with results and analyses of the testing of lobatamides A−D (**1**−**4**) in the NCI human tumor 60 cell-line screen. The lobatamides share a common core structure with the recently described salicylihalamides, which were isolated from a *Haliclona* sp. sponge. COMPARE analyses of the mean-graph differential cytotoxicity profiles of the lobatamides and the salicylihalamides showed high correlations with each other but not with members of the NCI's standard agents database. These compounds, therefore, appear to comprise a new mechanistic class, meriting further antitumor investigations.

Over the last 25 years tunicates have proved to be a consistent source of interesting and novel bioactive natural products.[2] Indeed, the first biologically active tunicate metabolites were isolated from the genus *Aplidium*.[3] Examples of metabolites previously isolated include prenylated quinones from *Aplidium* sp.,[3,4] *A. californicum*,[5] *A. costellatum*,[6] and *A. antillense*,[7] dimeric prenylated quinones from *Aplydium longithorax*,[8] chromenols from *A. solidum*,[9] a sphingosine derivative from an *Aplidium* sp.,[10] thiazole and imidazole metabolites from *A. placiferum*,[11] 1,2,3-trithiane derivatives from an *Aplidium* sp.,[12] alkaloids from an *Aplidium* sp.[13] and *A. pantherinium*,[14] and nucleosides from *A. multiplicatum*.[15]

We recently communicated[16] the isolation of two novel macrolides, lobatamides A (**1**) and B (**2**), from the tunicate *Aplidium lobatum* (Savigny); they are structurally unrelated to any of the numerous compounds isolated previously from other collections of *Aplidium*. We fully detail here the isolation and identification of **1** and **2**, as well as four additional members of the lobatamide family, lobatamides C−F (**3**−**6**), and biological evaluation of these compounds. The lobatamides have been isolated from three different shallow water collections of Australian *A. lobatum* (Q66C-2780, Q66C-2586, Q68C-2612), from *Aplidium* sp. collected during a trawling expedition at the Great Australian Bight, and from an unidentified, shallow water collection of a Philippine tunicate.

A typical isolation procedure for the lobatamides is as follows: The aqueous extract was first subjected to an EtOH precipitation procedure to remove anionic polysaccharides and large proteins.[17] The supernatant was partitioned between EtOAc and H_2O to yield EtOAc-soluble materials enriched in the lobatamides. This mixture was sequentially chromatographed using gel permeation (Sephadex LH-20, 1:1 MeOH−MeCN), VLC (amino-bonded phase, CH_2Cl_2−MeOH gradient, 100−

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† This paper is dedicated to the memory of a great friend and colleague Kenneth D. Paull (deceased, January 29, 1998), inventor of COMPARE.

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(2) (a) Ireland, C. M.; Roll, D. M.; Molinski, T. F.; McKee, T. C.; Zabriskie, T. M.; Swersey, J. C. In *Biomedical Importance of Marine Organisms*; Fautin, D. G., Ed.; California Academy of Sciences: San Francisco, CA, 1988; No. 13, pp 41−57. (b) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771−1791.

(3) Fenical, W. In Food-Drugs from the Sea, Proceedings 4th; Webber, H. H., Ruggieri, G. D., Eds.; Marine Technology Society: Washington, DC, 1974; pp 388−394.

(4) Guella, G.; Mancini, I.; Pietra, F. *J. Nat. Prod.* **1987**, *50*, 621−626.

(5) Howard, B. M.; Clarkson, K.; Berstein, R. L. *Tetrahedron Lett.* **1979**, 4449−4452.

(6) Targett, N. M.; Keeran, W. S. *J. Nat. Prod.* **1984**, *47*, 556−557.

(7) Benslimane, A. F.; Pouchus, Y. F.; Le Bouterff, J.; Verbist, J. F.; Roussakis, C.; Monniot, F. *J. Nat. Prod.* **1988**, *51*, 582−583.

(8) Fu, X.; Houssain, M. B.; van der Helm, D.; Schmitz, F. J. *J. Am. Chem. Soc.* **1994**, *116*, 12125−12126.

(9) Rochfort, S. J.; Metzger, R.; Hobbs, L.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 1217−1219.

(10) Carter, G. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 7441−7442.

(11) Arabshashi, L.; Schmitz, F. J. *Tetrahedron Lett.* **1988**, *29*, 1099−1102.

(12) Copp, B. R.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1989**, *30*, 3703−3706.

(13) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 825−832.

(14) Kim, J.; Pordesmo, E. O.; Toth, S. I.; Schmitz, F. J.; van Altena, I. *J. Nat. Prod.* **1993**, *56*, 1813−1816.

(15) (a) Kobayashi, J.; Doi, Y.; Ishibashi, M. *J. Org. Chem.* **1994**, *59*, 255−257. (b) Doi, Y.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 8651−8656.

(16) Galinis, D. L.; McKee, T. C.; Pannell, L. K.; Cardellina, J. H., II.; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8968−8969.

(17) Beutler, J. A.; McKee, T. C.; Fuller, R. W.; Tischler, M.; Cardellina, J. H., II.; McCloud, T. G.; Snader, K. M.; Boyd, M. R. *Antiviral Chem. Chemother.* **1993**, *4*, 167−172.

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Table 1. ^{13}C NMR Data (125 MHz, MeOH-d_4) for Lobatamides A−F (1−6)

C	1	2	3	4	5	6
1	170.0	170.0	169.9	169.9	170.0	170.0
2	122.3	122.3	122.2	122.3	122.3	122.2
3	156.7	156.7	156.6	156.6	156.7	156.6
4	114.4	114.4	114.2	114.4	114.4	114.4
5	131.8	131.8	131.7	131.9	131.9	131.9
6	120.9	120.9	120.8	120.8	120.9	120.9
7	141.2	141.2	141.1	141.2	141.2	141.2
8	33.1	33.1	33.0	33.1	33.1	33.1
9	125.6	125.6	125.5	125.7	125.7	125.7
10	139.4	139.4	139.4	139.4	139.4	139.4
11	73.3	73.3	73.2	73.4	73.4	73.4
12	135.0	135.0	134.9	137.6	137.6	137.4
13	132.7	132.7	132.6	128.1	128.1	128.2
14	73.8	73.8	73.7	78.4	78.1	78.4
16	171.9	171.8	171.7	172.1	172.1	172.1
17	38.9	38.9	38.8	38.9	38.9	38.9
18	73.0	73.0	72.9	73.1	73.1	73.1
19	35.6	35.6	35.5	35.6	35.6	35.6
20	109.9	110.2	110.2	109.8	110.2	110.2
21	126.9	126.8	126.9	126.9	126.9	126.9
23	164.2	164.2	164.1	164.3	164.2	164.2
24	126.1	127.9	130.2	126.1	127.9	130.2
25	135.6	127.5	135.5	135.9	127.6	135.5
26	148.7	144.4	149.3	148.7	144.5	149.4
28	62.7	62.5	62.7	62.7	62.6	62.8
29	19.6	19.6	19.5	19.6	19.7	19.7
30	20.2	20.2	20.2	64.2	64.2	64.2

90% CH_2Cl_2), Sanki CPC (5:7:4 $CHCl_3$−MeOH−H_2O, ascending mode, 1.7 mL/min), and finally repetitive HPLC (C_{18}, 3:7 MeCN−H_2O) to yield lobatamides A−F (0.5−3 mg/compound). The relatively low yields of the compounds likely are, in part, a reflection of their instability in both acid and base, observed during initial attempts at isolation.

As disclosed earlier,[16] lobatamides A and B (**1**, **2**) are macrolides which contain an unusual conjugated oxime methyl ether at the terminus of the side chain. The only difference between the two structures is the geometry of the oxime, *E* in **1** and *Z* in **2**. Support for the assignments came from the chemical shifts of both the C26 oxime carbon and its attached proton, which appeared downfield (δ_C 148.7; δ_H 8.95) for **1** relative to **2** (δ_C 144.4; δ_H 8.36 ppm) as did those of C25 (δ_C 135.6 and 127.5, for **1** and **2**, respectively).[18,19] In addition, the one-bond J_{CH} of the *E* oxime ether in **1** was 177 Hz but 190 Hz for **2**, values which were similar to the calculated and experimentally observed coupling constants of oximes.[20]

The third compound, lobatamide C (**3**), was isolated and shown by HRFABMS to be isomeric with lobatamides A and B (**1**, **2**), namely $C_{27}H_{32}N_2O_8$. As with **1** and **2**, the presence of three exchangeable protons was indicated by a CIMS deuterium exchange experiment using ND_3 as the ionizing agent.[21] The structural similarities among the three compounds were clearly evident from both the ^{13}C and ^{1}H NMR spectra (Tables 1 and 2). The ^{13}C NMR spectrum of **3** contained signals for all 27 carbons, including the following: 2 ester carbonyls (δ 171.7, 169.9); an amide carbonyl (δ 164.1); 15 sp^2 carbons, 14 of which were accounted for by a phenyl ring and 4 olefins; 3 oxygenated methine carbons (δ 73.7, 73.2, 72.9); 3 methylenes (δ 33.0, 35.5, 38.8); 3 methyl groups [δ 62.7 (OCH_3), 20.2, 19.5].

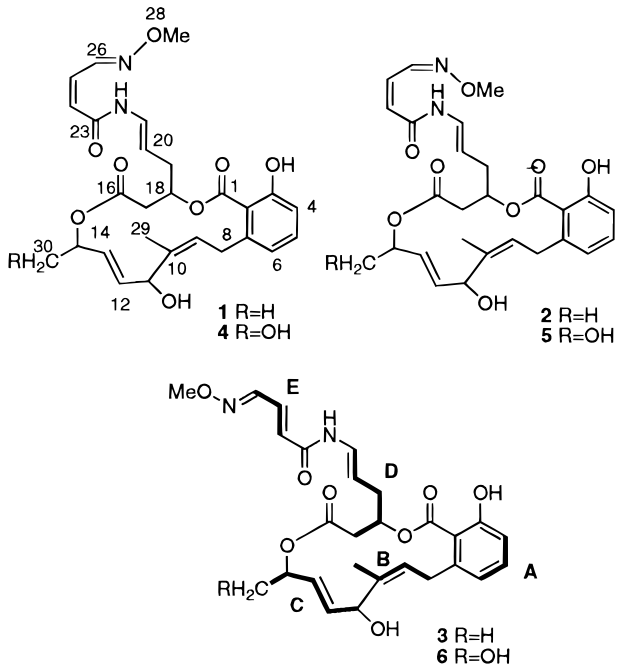

Figure 1. Structures of lobatamides A−F (**1**−**6**). The bold lines on **3** indicate discrete spin systems identified by NMR experiments. The letter adjacent to each system is used to identify an individual spin system discussed in the text.

On the basis of a standard series of one- and two-dimensional NMR experiments, which included HMQC and HMBC, the five spin systems shown in Figure 1 could be identified for lobatamide C. Of the five spin systems defined, only one system (**E**) was different from those found in lobatamides A and B (**1**, **2**). This system contained a trans olefin on the basis of the 15.1 Hz coupling constant between H24 and H25. This coupling constant was 11.7 Hz for both lobatamides A and B. For lobatamide C (**3**) the oxime methyl ether was assigned as *E* on the basis of the carbon chemical shifts for C26 and C25 at δ_C 149.3 and 135.5, respectively; in addition, the one bond J_{CH} of C26 was 175 Hz. These spectral data closely match those of lobatamide A.[16]

Fragments **A** and **B** were connected through C7, based on HMBC correlations (HMBC experiments optimized for J_{nCH} 8.3 and 5.5 Hz) of that carbon to protons at δ 7.14 (H5) and δ 3.21 (H8a). Additional correlations supporting this connection were those between H6 and C8, and H8 and C2. Correlations from C10 to H11 and H12, along with those observed to H29 from C9, C10, and C11 provided the connection between fragments **B** and **C**. The correlation between H14 and the ester carbonyl at δ 171.7 (C16), which was further correlated to both protons on C17 (δ 2.60, 2.68), as well as H18, provided the connection between fragments **C** and **D**. H18 (δ 5.60) was further correlated to the second ester carbonyl at δ 169.9 (C1), which was previously linked to fragment **A** at C2. Finally, fragments **D** and **E** were linked through an amide bond on the basis of the observed correlations between the carbonyl at δ 164.1 (C23) and H24 and H25. No correlation between H21 and C23 was observed in the HMBC experiments with **3**; however, the presence of the amide bond was supported by the similarities of

(18) Gordon, M. S.; Sojka, S. A.; Krause, J. G. *J. Org. Chem.* **1984**, *49*, 97−100.

(19) Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tables of Spectral Data for Structure Determination for Organic Compounds*; Springer-Verlag: Berlin, 1983.

(20) Maciel, G. E.; McIver, J. W., Jr.; Ostlund, N. S.; Pople, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 1−11.

(21) Daly, J. W.; Spande, T. F.; Whitaker, N.; Highet, R. J.; Feigl, D.; Nishimori, N.; Toykuyama, T.; Myers, C. W. *J. Nat. Prod.* **1986**, *49*, 265−280.

Table 2. ^{1}H NMR Data (500 MHz, MeOH-d_4) for Lobatamides A−F (1−6)

	^{1}H δ (mult, J in Hz)					
no.	**1**	**2**	**3**	**4**	**5**	**6**
4	6.68 (d, 7.8)	6.68 (d, 7.8)	6.68 (d, 8.3)	6.68 (d, 7.8)	6.68 (d, 8.3)	6.68 (d, 7.8)
5	7.14 (dd, 7.8, 7.8)	7.14 (dd, 7.8, 7.8)	7.14 (dd, 7.8, 8.3)[a]	7.14 (dd, 7.8, 7.8)	7.14 (dd, 7.8,8.3)	7.14 (dd, 7.3, 7.8)[b]
6	6.63 (d, 7.8)	6.63 (d, 7.8)	6.63 (d, 7.8)	6.63 (d, 7.8)	6.63 (d, 7.8)	6.63 (d, 7.3)
8a	3.21 (dd, 8.3, 17.1)	3.21 (dd, 8.8, 18.1)	3.21 (dd, 8.8, 17.6)	3.25 (dd, 8.8, 17.1)	3.25 (dd, 9.3, 17.6)	3.21 (dd, 8.8, 17.6)
8b	2.93 (br d, 17.1)	2.93 (br d, 18.1)	2.95 (br d, 17.6)	2.96 (br d, 17.1)	2.95 (br d, 17.6)	2.95 (br d, 17.6)
9	5.17 (m)	5.17 (m)	5.17 (m)	5.20 (m)	5.18 (m)	5.17 (m)
11	4.78 (d, 8.8)	4.78 (d, 8.8)	4.79 (d, 8.8)	4.80 (d, 8.3)	4.79 (d, 8.8)	4.79 (d, 8.8)
12	5.66 (dd, 8.8, 15.1)	5.66 (dd, 8.8, 15.1)	5.67 (dd, 8.8, 15.1)	5.76 (dd, 6.6, 15.6)	5.75 (dd, 8.8, 15.1)	5.78 (dd, 8.8, 15.6)
13	5.50 (dd, 8.3, 15.1)	5.50 (dd, 8.3, 15.1)	5.50 (dd, 8.8, 15.1)	5.48 (dd, 9.3, 15.6)	5.50 (dd, 9.3, 15.1)	5.50 (dd, 8.8, 15.6)
14	5.23 (dq, 6.3, 8.3)	5.23 (dq, 6.8, 8.3)	5.23 (dq, 8.8, 6.4)	5.16 (m)	5.16 (m)	5.14 (m)
17a	2.67 (dd, 2.1, 16.6)	2.67 (dd, 2.4, 16.6)	2.68 (dd, 2.4, 16.6)	2.73 (dd, 2.0, 16.6)	2.73 (dd, 2.4, 16.6)	2.73 (dd, 2.4, 16.6)
17b	2.59 (dd, 10.7, 16.6)	2.59 (dd, 10.7, 16.6)	2.60 (dd, 10.8, 16.6)	2.67 (dd, 10.7, 17.1)	2.67 (dd, 10.2, 16.6)	2.67 (dd, 10.7, 16.6)
18	5.58 (m)	5.58 (m)	5.60 (m)	5.58 (m)	5.60 (m)	5.61 (m)
19	2.48 (2H, dd, 6.3, 7.3)	2.48 (2H, br t, 6.8)	2.48 (2H, m)	2.48 (2H, br t, 6.8)	2.48 (2H, br t, 6.8)	2.48 (2H, br t, 6.8)
20	5.34 (dt, 7.8, 14.2)	5.34 (m)	5.37 (dt, 7.8, 14.6)	5.34 (dt, 7.8, 14.2)	5.35 (dt, 7.3, 14.2)	5.37 (dt, 7.3, 14.2)
21	6.82 (d, 14.2)	6.82 (d, 14.2)	6.87 (d, 14.6)	6.82 (d, 14.6)	6.83 (d, 14.2)	6.87 (d, 14.2)
24	6.04 (d, 11.7)	6.05 (d, 11.7)	6.28 (d, 15.1)	6.05 (d, 11.2)	6.05 (d, 11.7)	6.28 (d, 15.6)
25	6.45 (dd, 11.2, 11.7)	7.04 (dd, 9.8, 11.7)	7.14 (dd, 10.2, 15.1)[a]	6.49 (dd, 10.2, 11.2)	7.03 (dd, 9.8, 11.7)	7.13 (dd, 9.8, 15.6)[b]
26	8.95 (d, 11.2)	8.36 (d, 9.8)	7.86 (d, 10.2)	8.95 (d, 10.2)	8.36 (d, 9.8)	7.85 (d, 9.8)
28	3.91 (3H, s)	3.91 (3H, s)	3.91 (3H, s)	3.89 (3H, s)	3.89 (3H, s)	3.91 (3H, s)
29	1.79 (3H, s)	1.79 (3H, s)	1.79 (3H, s)	1.79 (3H, s)	1.80 (3H, s)	1.78 (3H, s)
30a	1.35 (3H, d, 6.3)	1.34 (3H, d, 6.8)	1.34 (3H, d, 6.3)	3.70 (dd, 7.8, 12.1)	3.70(dd, 7.3, 12.2)	3.70 (dd, 7.3, 11.7)
30b				3.65 (dd, 3.9, 12.1)	3.65 (dd, 3.9, 12.2)	3.65 (dd, 3.9, 11.7)

[a,b] Overlapping multiplets.

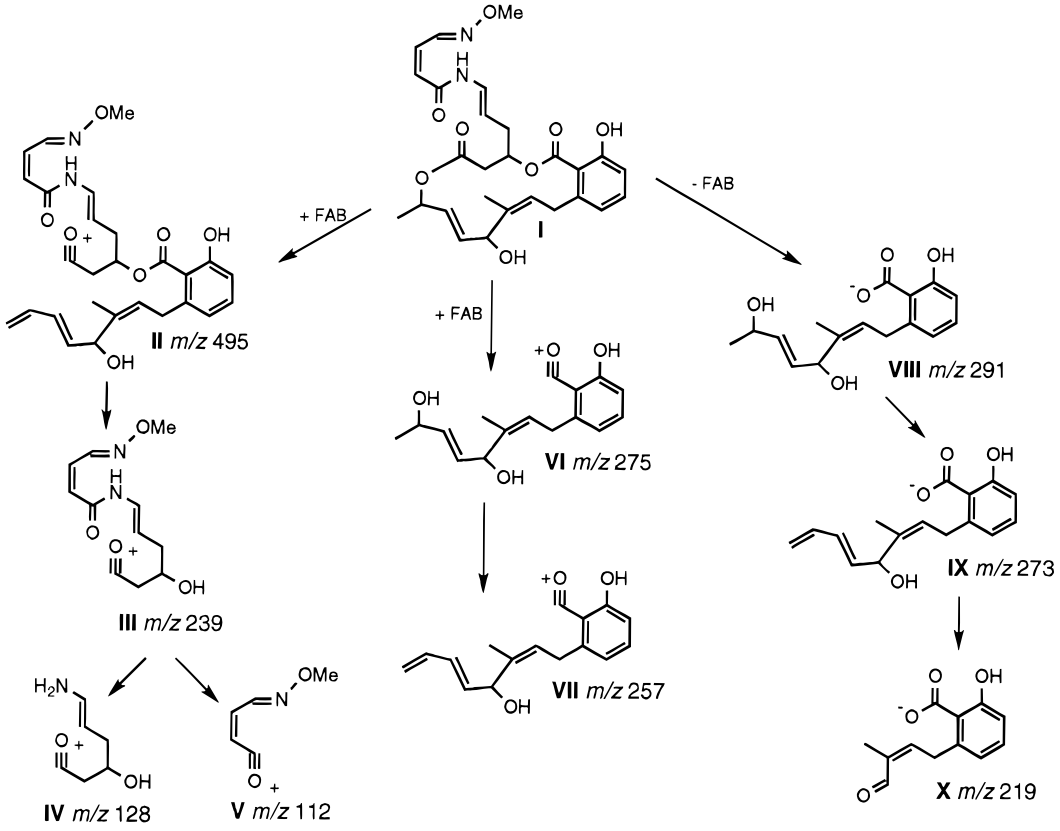

Figure 2. Deduced fragmentation pattern of lobatamides A−C (**1**−**3**) in both positive and negative FAB, using linked scan analyses of the parent ions.

both the ^{1}H and ^{13}C NMR spectra and the linked scan mass spectrometry studies. Thus, HMBC experiments led to the gross structure of lobatamide C (**3**).

As with lobatamides A and B, the deduced structure for lobatamide C was supported by linked scan mass spectrometry. In all three cases, the identification of the same molecular formula and observation of the same fragmentations (see Figure 2) supported the three structures (**1**−**3**) as geometric isomers. Compounds **1**−**3** fragmented to give several structurally significant ions (Figure 2), the sequence of which was determined by linked scan analyses of the fragment ions. In positive ion mode, elimination of a water molecule from the pseudomolecular (MH^+) ion dominated the upper mass range to give a peak at *m/z* 495 (**II**). Although more than one structure can be drawn for this ion, **II** is based on subsequent fragmentation and deuterium exchange data. Cleavage of the second ester bond yielded a dominant

ion at *m/z* 239 (**III**), characteristic of the upper half of the molecule. This further fragmented to yield ions at *m/z* 128 (**IV**) and *m/z* 112 (**V**). This latter fragment (**V**, *m/z* 112) strongly supported placement of the methoxyl on an oxime. Initial fragmentation at the other ester bond yielded fragment ions at *m/z* 275 (**VI**) and then *m/z* 257 (**VII**). In negative ion mode, fragmentation was dominated by cleavage at both ester bonds to yield ions at *m/z* 291 (**VIII**), *m/z* 273 (**IX**), and *m/z* 219 (**X**), further characterizing the macrocyclic ring. Upon acetylation, compound **1** yielded a diacetate, *m/z* 596, which, on fragmentation in positive ion mode, gave further support to structure **1** (fragment ions comparable to **II**, **VI**, and **VII** showed two acetylations, while analogous structures **III**−**V** showed no change).

The three additional isolated compounds, lobatamides D−F (**4**−**6**), all had a molecular formula of $C_{27}H_{32}N_2O_9$, indicating the presence of one more oxygen than lobatamides A−C. Deuterium-exchange CIMS experiments confirmed that the extra oxygen could be accounted for by an additional hydroxyl group in compounds **4**−**6**. For lobatamide D (**4**), a standard set of one- and two-dimensional NMR experiments was obtained to identify the five spin systems present; of these, four (**A**, **B**, **D**, and **E** of Figure 1) were identical to those of lobatamide A (**1**). Fragment **C**, however, terminated in a hydroxy methylene (δ_C 64.2; δ_H 3.70, dd, J = 12.1, 7.8 Hz; 3.65, dd, J = 12.1, 3.9 Hz) rather than a methyl group. Linked scan mass spectrometry studies of lobatamide D indicated that several of the fragment ions differed by 16 amu relative to those of **1**−**3**. These ions included **II** (to *m/z* 511), **VI** (to *m/z* 291), **VII** (to *m/z* 273), **VIII** (to *m/z* 307), and **IX** (to *m/z* 289). In addition, fragment **VII** lost the elements of water to yield an ion at *m/z* 255, thus establishing the C30 location of the extra hydroxyl group in **4**. The carbon and proton chemical shifts and J_{CH} values closely matched those reported for **1**, indicating that both compounds possessed the same geometries at the $\Delta^{24,25}$-olefin and the oxime bonds.

Comparison of the ^{1}H and ^{13}C NMR spectra obtained for lobatamides D (**4**) and E (**5**) suggested they differed only in the geometry of the oxime methyl ether functionality. The chemical shift for the oxime carbon (C26) in **5** appeared upfield relative to that for **4** (144.5 and 148.7 ppm, respectively), as was that for C25 (127.6 and 135.9 ppm for **5** and **4**, respectively). These differences in the carbon spectra were paralleled by an analogous upfield shift of the signal for H26 (8.36 ppm for **5** relative to 8.95 ppm for **4**). In addition, the one-bond heteronuclear coupling constant for C26 in **5** was 190 Hz. These data indicated that the oxime methyl ether was of *Z*-geometry, as was the case with lobatamide B (**2**). A comparison of the spectral data for **2** and **5** showed a close correlation between the chemical shifts in both the ^{1}H and ^{13}C spectra, supporting the structure of lobatamide E (**5**).

A comparison of the ^{1}H and ^{13}C spectra and a series of NMR experiments confirmed the gross structure of lobatamide F (**6**). As with lobatamide C (**3**), the C24−C25 olefin had *E* geometry on the basis of the 15.6 Hz coupling constant. The consistent coupling constant of 10 Hz between H25 and H26, as well as the NOE observed between the two protons, indicated that the olefin−oxime bonds were in an s-cis relationship. Finally, the carbon chemical shifts of C26 (149.4 ppm) and C25 (135.5 ppm) corresponded closely with those of lobatamides A, C, and D and were downfield of the C25 and C26 shifts of lobatamides B and E. In addition, the one-bond heteronuclear coupling constant of 178 Hz was consistent with those of the other lobatamides possessing *E*-geometry at the oxime bond.

A mixture of the lobatamides A and B (9.5 mg) was also obtained from an unidentified tunicate collected near the Cuyo Island in the Philippines. The small collection (143 g frozen, wet weight) of the tunicate had the general physical characteristics of an *Aplidium* sp., appearing as a thick, fleshy, encrusting black and orange mottled sheet with orange zooids. The lobatamides were isolated by bioassay guided fractionation using cytotoxicity against HCT 116 cells as a marker.

The overall structures of the lobatamides are unique, due primarily to the unusual enamide−oxime methyl ether structure which terminates the side chain of the macrolide. We have concluded that lobatamides A, B, D, and E are the same compounds as those originally isolated[31] and named aplidites A, B, C, and D, respectively, from an *Aplidium* sp. from the Great Australian Bight. Individual comparisons of the spectroanalytical data of the different samples of **1**−**4** isolated by our collaborating laboratories justify a revision of the structures originally depicted for aplidites A−D and support the structures shown herein as **1**−**4**. In addition to revising both the structures and trivial nomenclature of aplidites A−D, we also take this opportunity to revise the earlier structures and nomenclature reported[31] for aplidites E−G, to those for lobatamides G−I (**7**−**9**) (Chart 1) as shown. Prior to the elucidation of the lobatamides, there had been no reports of any oxime-containing metabolites from tunicates. However, oxime-containing compounds have been isolated by several groups from sponges; examples include *Ianthella* spp. (bastadins)[22] and *Psammaplysilla* spp. (purealins[23] and bromotyrosine alkaloids[24]) and *Niphates* sp. (niphatesines).[25] There has also been one oxime-containing metabolite reported from the green alga *Acrosiphonia coalita*[26] and one from the dinoflagellate *Gymnodinium breve*.[27]

Lobatamides A−D (**1**−**4**) were tested in the NCI's 60 cell line human tumor screen.[28,29] The compounds showed a characteristic pattern of differential cytotoxicity and

(22) For examples see: (a) Pordesimo, E. O.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704−4710. (b) Butler, M. S.; Lim, T. K.; Capon, R. J.; Hammond, L. S. *Aust. J. Chem.* **1991**, *44*, 287−296. (c) Gulavita, N.; Wright, A. E.; McCarthy, P. J.; Pomponi, S. A.; Kelly-Borges, M.; Chin, M.; Sills, M. A. *J. Nat. Prod.* **1993**, *56*, 1613−1617.

(23) Nakamura, H.; Wu, H.; Kobayashi, J.; Nakamura, Y.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1985**, *26*, 4517−4520.

(24) For examples, see: (a) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 3229−3232. (b) Kobayashi, J.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. *Tetrahedron* **1991**, *47*, 6617−6620. (c) Yagi, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749−3754. (d) Jurek, J.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1993**, *56*, 1609−1612.

(25) Kobayashi, J.; Zeng, C. M.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Mikami, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1291−1294.

(26) Bernart, M. W.; Whatley, G. G.; Gerwick, W. H. *J. Nat. Prod.* **1993**, *56*, 245−259.

(27) Alam, M.; Sanduja, R.; Hossain, M. B.; van der Helm, D. *J. Am. Chem. Soc.* **1982**, *104*, 5232−5234.

(28) Boyd, M. R.; Paull, K. D. *Drug Devel. Res.* **1995**, *34*, 91−109.

(29) Boyd, M. R. In *Cancer: Principles and Practice of Oncology Updates*; DeVita, V. T., Jr., Hellman, S., Rosenberg, S. A., Eds.; Lipincott: Philadelphia, PA, 1989; Vol. 3, No. 10, pp 1−12.

(30) Erickson, K. L.; Beutler, J. A.; Cardellina, J. H, II; Boyd, M. R. *J. Org. Chem.*, **1997**, *62*, 8188−8192.

(31) Murray, L.; Lim, T. K.; Currie, G.; Capon, R. J. *Aust J. Chem.* **1995**, *48*, 1253−1266.

Chart 1

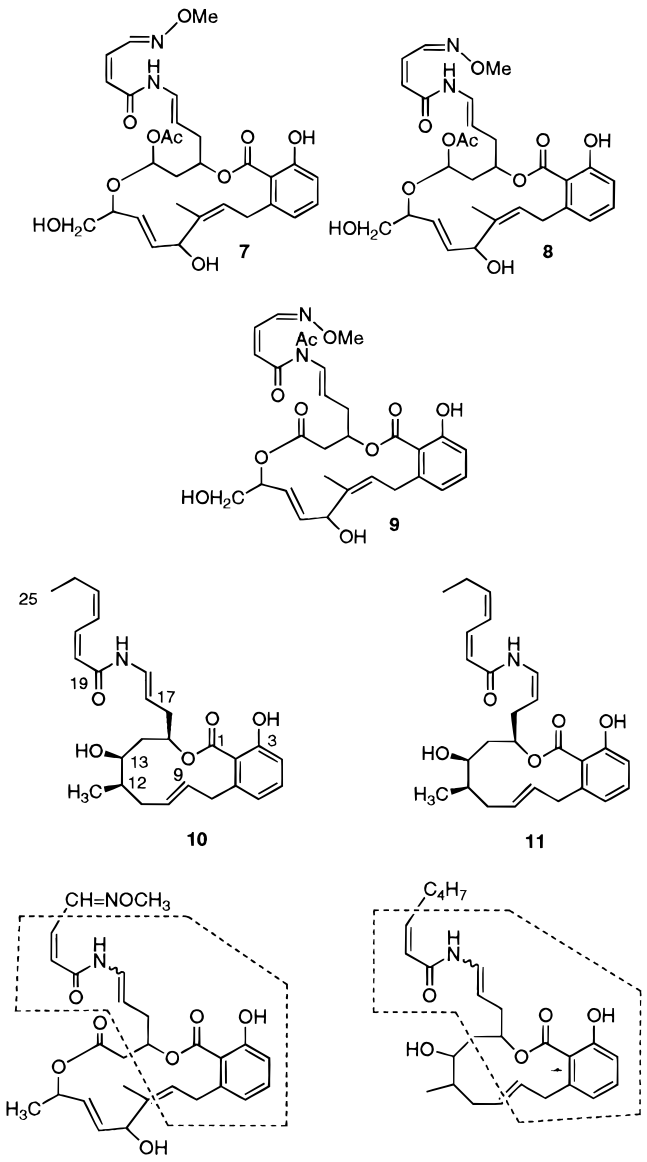

Figure 3. Common core structural component shared by the lobatamides and the salicylihalamides. The broken lines identify this common core structure as encompassed within lobatamide A (left) and salicylihalamide A (right).

were approximately equipotent (e.g., mean panel GI_{50}'s ~ 1.6 nM). COMPARE pattern-recognition analyses of the mean-graph profiles[29,30] of **1** did not reveal any significant correlations to the profiles of reference antitumor compounds contained in the NCI's standard agent database. This suggests that the lobatamides may act by a novel mechanism of action since their activity profiles did not match (correlate using COMPARE) compounds whose mechanism of action are known. The differential cytotoxicity profiles of the lobatamides did, however, show high (≥0.7) COMPARE correlations among themselves, as well as with the salicylihalamides (**10**, **11**), recently isolated from a sponge (*Haliclona* sp.).[30] This is perhaps not surprising, given certain structural similarities within the two compound families. While the size of the macrolide ring differs (12 for the salicylihalamides and 15 for the lobatamides), both families contain the common core structure depicted in Figure 3. The core differs only in how the enamide side chain terminates, ending in the salicylihalamides as a heptadienamide and in the lobatamides as a conjugated olefin oxime methyl ether.

Lobatamide A (**1**)[16] is identical to the structure of YM-75518, recently reported by Suzumura and co-workers[32] from a terrestrial *Pseudomonas* sp. cultured from an Indonesian soil sample. The occurrence of this identical compound in collections of different tunicates from distinct geographical locations and from a terrestrial pseudomonad suggests the possibility of their biosynthesis in the tunicates by associated microorganisms or by accumulation through dietary intake. This argument might be further supported by the isolation of the biosynthetically related salicylihalamides from the sponge *Haliclona* sp.[30] Definitive assessments of such possibilities will require the ability to separate and culture the associated microorganisms in an environment that mimics that of the host tunicate. Also, an intriguing alternative to consider may be that tunicates, like some microorganisms, are able to acquire the biosynthetic machinery from other organisms to expand and/or acquire secondary metabolite arsenals. Eventually, comparisons of genomic information from *Pseudomonas* sp. and *Aplidium lobatum* may enable comparisons of the encoded biosynthetic machinery pertinent to these questions.

Experimental Section

Isolation of the Lobatamides. Shallow Water Australian Collections. These compounds have been isolated from three different *Aplidium lobatum* samples (Q66C-2780, Q66C-2586, Q68C-2612) collected under contract to the National Cancer Institute. For example, Q66C-2612 was collected off the southwestern coast of Australia due west of Hillary Boat Harbor at −6 m and identified as *Aplidium lobatum* by Patricia Kott of Queensland Museum. Vouchers are maintained at the Australian Institute of Marine Science and the Smithsonian Institution. A typical isolation procedure for the lobatamides is as follows.

A sample (642 g wet weight) of the *A. lobatum* specimen coded Q66C-2612 was frozen immediately after collection until extraction as described.[33] The aqueous extract (13.8 g) was taken through an EtOH precipitation procedure to remove large proteins and sulfated polysaccharides.[17] The supernatant was partitioned between EtOAc and H_2O to yield an EtOAc-soluble fraction enriched with the cytotoxic compounds of interest. This material was sequentially chromatographed using gel permeation (Sephadex LH-20, 1:1 MeOH−MeCN), followed by VLC (amino-bonded phase, CH_2Cl_2−MeOH gradient, 100−90% CH_2Cl_2), Sanki CPC (5:7:4 CH_3Cl−MeOH−H_2O, ascending mode, 1.7 mL/min), and finally repeated HPLC (RP18, 3:7 MeCN−H_2O) to yield lobatamides A (1.1 mg, 0.008%), B (1.4 mg, 0.01%), C (0.6 mg, 0.004%), D (1.0 mg, 0.007 %), E (2.8 mg, 0.02%), and F (2.6 mg, 0.02%). Small amounts of **1**−**6** were also present in the organic crude extracts.

Deep Water Australian Collections. The deep water collection of *Aplidium* sp. from the Great Australian Bight and the isolation of compounds from this collection have been described.[31]

Philippine Collections. The unidentified tunicate was collected in April 1993, in collaboration with Dr. Hilconida Calumpong, Silliman University, off the coast of Coccoro Island, Cuyo Islands, the Philippines. The black and orange mottled tunicate with orange zooids was 1.5 cm thick and encrusting and shaped like fleshy leaves. No voucher sample

(32) Suzumura, K.; Takahashi, I.; Matsumodo, H.; Nagai, K.; Setiawan, B.; Rantiatmodjo, R. M.; Suzuki, K.; Nagano, N. *Tetrahedron Lett.* **1997**, *38*, 7573−7576.

(33) Dai, J.-R.; Hallock, Y. F.; Cardellina, J. H., II; Gray, G. N.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 860−865.

is available. The frozen ascidian (143.9 g frozen, wet weight) was ground with MeOH (300 mL) in a blender (Osterizer). The ground sample was exhaustively extracted with H_2O, MeOH, and $CHCl_3$ (and combined) to obtain a crude extract (6.4 g), which was dissolved in MeOH (90 mL) and H_2O (10 mL) and was extracted with hexane (6 × 100 mL). H_2O (20 mL) was added to the original solution, which was then extracted with $CHCl_3$ (3 × 100 mL). The $CHCl_3$ fraction was then back-extracted with H_2O (2 × 70 mL). Three fractions were obtained from this liquid−liquid partitioning scheme: hexane, $CHCl_3$−MeOH, and MeOH−H_2O solubles. Each of these fractions was cytotoxic toward HCT 116 cells at 5 μg/mL. The $CHCl_3$−MeOH soluble fraction was subjected to silica gel VLC using a $CHCl_3$−MeOH gradient. The active fraction eluted with ca. 5% MeOH in $CHCl_3$. Silica gel flash chromatography ($CHCl_3$−MeOH gradient) of this active fraction yielded a mixture of lobatamides A and B (**1**, **2**, 9.5 mg, 0.07%).

Lobatamide A (1): $[\alpha]_D$ −7.9° (*c* 0.24, MeOH); UV (MeOH) λ_{max} 273 (log ϵ 4.22) nm; IR (film) ν_{max} 3590−3128 (br), 2974, 2933, 1739, 1656, 1523, 1461, 1451, 1374, 1266, 1215, 1169, 1113, 1042 cm^{-1}; HRFABMS (noba) MH$^+$ *m/z* 513.2257, calcd for $C_{27}H_{33}N_2O_8$ 513.2255; FABMS (noba) *m/z* 535 (M+ Na$^+$, 8%), 513 (MH$^+$, 16), 495 (15), 460 (20), 239 (22), 138 (100), 107 (83).

Linked Scan Mass Spectrometry. Linked-scan FABMS spectra were run on a JEOL SX102 spectrometer using a using a 10 kV xenon gun to desorb the samples from a magic bullet matrix (5:1 DTT−DTE). Spectra were run in both positive and negative ionization conditions. Fragmentation analyses were performed by B/E linked scans of the parent and fragmentation ions. Where necessary, a helium collision gas was used in the first field free region to enhance this fragmentation. Exchange spectra were performed under chemical ionization (CI) conditions on a Finnigan 4500 spectrometer using the direct exposure probe. Exchanges were determined by comparing spectra obtained using ND_3 as the reagent gas with those from NH_3. HRFABMS data (*m/e*) for structurally significant fragments of lobatamide A (**1**): **II**, 495.2115 for $C_{27}H_{31}N_2O_7$, calcd 495.2131, 3 exchangeable protons; **III**, 239.1021 for $C_{11}H_{15}N_2O_4$, calcd 239.1032, 2 exchangeable protons; **V**, 112.0402 for $C_5H_6NO_2$, calcd 112.0399; **VI**, seen in CI only, 3 exchangeable protons; **VII**, 257.1129 for $C_{16}H_{17}O_3$, calcd 257.1137, 2 exchangeable protons; **VIII**, 291.1231 for $C_{16}H_{19}O_5$, calcd 291.1231; **IX**, 273.1118 for $C_{16}H_{17}O_4$, calcd 273.1127; **X**, 219.0650 for $C_{12}H_{11}O_4$, calcd 219.0657.

Lobatamide B (2): $[\alpha]_D$ −15.0° (*c* 0.03, MeOH); UV (MeOH) λ_{max} 281 (log ϵ 4.55); IR (film) ν_{max} 3580−3118 (br), 3056, 2974, 2933, 1733, 1651, 1605, 1584, 1528, 1467, 1451, 1267, 1221, 1175, 1113, 1046 cm^{-1}; HRFABMS (magic bullet) MH$^+$ *m/z* 513.2244, calcd for $C_{27}H_{33}N_2O_8$ 513.2255; FABMS (glyc) *m/z* 513 (MH$^+$, 10%), 495 (10), 461 (8), 279 (55), 239 (23), 177 (25), 153 (100), 135 (100).

Lobatamide C (3): $[\alpha]_D$ −15.5° (*c* 0.113, MeOH); UV (MeOH) λ_{max} 280 (log ϵ 4.04) nm; IR (film) ν_{max} 3590−3108 (br), 3067, 2975, 2933, 1733, 1656, 1616, 1584, 1467, 1451, 1354, 1267, 1215, 1175, 1113, 1042, 959, 790 cm^{-1}; HRFABMS (magic bullet) MNa$^+$ *m/z* 535.2065, calcd for $C_{27}H_{33}N_2O_8$ 513.2255; FABMS (m-bullet) *m/z* 551 (MNa$^+$, 55%), 535 (60), 495 (20), 257 (32), 239 (50), 193 (100).

Lobatamide D (4): $[\alpha]_D$ −35.0° (*c* 0.08, MeOH); UV (MeOH) λ_{max} 281 (log ϵ 4.23); IR (film) ν_{max} 3593−3123 (br), 2924, 1738, 1650, 1607, 1529, 1464, 1450, 1269, 1218, 1168, 1117, 1042, 964, 755 cm^{-1}; HRFABMS (m-bullet) MH$^+$ *m/z* 529.2164, calcd for $C_{27}H_{33}N_2O_9$ 529.2186; FABMS (noba) *m/z* 551 (MNa$^+$, 100%), 529 (6), 511 (10), 239 (12).

Lobatamide E (5): $[\alpha]_D$ −26.7° (*c* 0.06, MeOH); UV (MeOH) λ_{max} 282 (log ϵ 4.25); IR (film) ν_{max} 3591−3110 (br), 2924, 1734, 1652, 1539, 1269, 1046, 668 cm^{-1}; HRFABMS (m-bullet) MH$^+$ *m/z* 529.2184, calcd for $C_{27}H_{33}N_2O_9$ 529.2186; FABMS (glyc) *m/z* 551 (MNa$^+$, 5%), 511 (17), 239 (33), 217 (30), 112 (100).

Lobatamide F (6): $[\alpha]_D$ −19.2° (*c* 0.067, MeOH); UV (MeOH) λ_{max} 280 (log ϵ 4.17); IR (film) ν_{max} 3591−3108 (br), 2912, 1737, 1657, 1607, 1525, 1481, 1268, 1214, 1167, 1114, 1042, 973, 755, 668 cm^{-1}; HRFABMS (noba) MNa$^+$ *m/z* 551.2015, calcd for $C_{27}H_{32}N_2O_9Na$ 551.2006; FABMS (magic bullet) *m/z* 551 (MNa$^+$, 50), 511 (20), 301 (40), 239 (10), 112 (100).

Cytotoxicity Profiles in the NCI 60-Cell Line Screen. Lobatamides A−D (**1**−**4**) were tested in triplicate in the NCI in vitro screen;[29,30] mean graphs were constructed and averaged. The averaged, individual negative log GI_{50} values comprising the GI_{50} mean graph, along with the respective subpanel and cell-line identifiers are as follows for **1**: [leukemia] CCRF-CEM (9.21), HL-60(TB) (9.59), K-562 (8.72), MOLT-4 (9.05), RPMI-8226 (8.89), SR (9.59); [nonsmall cell lung] A549/ATCC (9.00), EKVX (8.92), HOP-62 (9.49), HOP-92 (8.85), NCI-H226 (9.48), NCI-H23 (8.36), NCI-H322M (8.31), NCI-H460 (9.15), NCI-H522 (8.70); [colon] COLO 205 (9.12), HCT-116 (9.11), HCT-15 (8.60), HT29 (9.35), KM12 (9.04), SW-620 (8.77); [CNS] SF-268 (9.32), SF-295 (9.44), SF539 (9.13), SNB-19 (>7.00), SNB-75 (8.34), U251 (9.00); [melanoma] LOX IMVI (9.70), MALME-3M (8.52), M14 (9.57), SK-MEL-2 (8.89), SK-MEL-28 (8.12), SK-MEL-5 (9.60), UACC-257 (9.11), UACC-62 (9.32); [ovarian] IGROV1 (8.60), OVCAR-3 (8.96), OVCAR-5 (7.51), OVCAR-8 (9.22), SK-OV-3 (>7.00); [renal] 786-0 (9.39), A498 (7.42), ACHN (9.10), CAKI-1 (8.70), RXF-393 (9.68), SN12C (7.49), TK-10 (7.59), UO-31 (8.82); [prostate] PC-3 (8.29), DU-145 (8.27); [breast] MCF7 (8.14), MCF7/ADR-RES (8.60), MDA-MB-231/ATCC (7.77), HS578T (8.57), MDA-MB-435 (9.03), MDA-N (8.80), BT-549 (9.30), T-47D (8.70).

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Supporting Information Available: ^{1}H NMR spectra of lobatamides A−F and ^{13}C NMR spectra of lobatamides A−C (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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