(c 1.0, $CHCl_3$); IR 3300, 2940, 2870, 1450, 1390, 1365, 1140, 1080, 1015, 745 cm^{-1}; ^1H NMR ($CDCl_3$) 1.96 (br s, 1 H), 3.73 (dd, J = 11.6, 5.2 Hz, 1 H), 3.92 (dd, J = 11.6, 7.2 Hz, 1 H), 4.03 (ddd, J = 1.7, 1.6, 1.4, 1 H), 4.22 (ddd, J = 7.0, 5.2, 1.6 Hz, 1 H), 4.31 (dd, J = 12.7, 1.7 Hz, 1 H), 4.37 (dd, J = 12.7, 1.4 Hz, 1 H), 5.57 (s, 1 H), 7.3-7.6 (m, 5 H); ^{13}C NMR δ 53.7, 63.4, 72.3, 78.7, 101.8, 126.2, 128.3, 129.3, 137.3. Anal. Calcd for $C_{11}H_{13}ClO_3$: C, 57.78; H, 5.73. Found: C, 57.74; H, 5.63.

(2*S*,4*R*,5*R*)-5-Chloro-4-[(*N*-benzylcarbamyl)hydroxymethyl]-2-phenyl-1,3-dioxane (21). Benzylidene **20** (229 mg, 1.0 mmol), benzyl isocyanate (148 μL, 1.2 mmol), and diisopropylethylamine (260 μL, 1.5 mmol) were stirred in benzene (5 mL) at 55 °C for 5 h. The solution was concentrated and chromatographed on silica gel [ethyl acetate/hexane (1:1.75)]: R_f 0.35; mp 171-172 °C; $[\alpha]^{25}_D$ +23.1° (c 0.6, $CHCl_3$); IR 3340, 2980, 2870, 1700, 1540, 1270, 1140, 1080, 755, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.01 (s, 1 H), 4.32-4.43 (m, 7 H), 5.56 (s, 1 H), 7.2-7.4 (m, 8 H), 7.55-7.7 (m, 2 H); ^{13}C NMR δ 45.2, 53.7, 65.2, 72.2, 76.5, 101.8, 126.3, 127.6, 128.3, 128.7, 129.2, 137.5, 139.0, 156.1. Anal. Calcd for $C_{19}H_{20}ClNO_4$: C, 63.07; H, 5.57; N, 3.87. Found: C, 63.30; H, 5.61; N, 4.06.

L-*threo*-Fluoromalic Acid. Difluorofumaric acid (1.22 g, 8 mmol) was dissolved in water (50 mL), and NaOH (0.64 g, 16 mmol) was added to neutralize the solution. Sodium formate (0.58 g, 8.5 mmol) was added, and the solution was deoxygenated. The pH was adjusted to 7.2, and NADH (125 mg, 0.16 mmol) was added, followed by formate dehydrogenase (375 mg, 250 U), malate dehydrogenase (43 μL, 500 U), and fumarase (0.8 mL, 1250 U, fumarate as substrate). The solution was stirred under nitrogen with the pH maintained at 7.1-7.2 by using a pH controller operating a peristaltic pump adding 1 N HCl or 1 N NaOH to the reaction mixture. Progress of the reaction was monitored at 230 nm. After 24 h the mixture was acidified to pH 1 with concentrated HCl and continuously extracted with ether. The ether extracts were evaporated and dried in vacuo to a white solid; 1.35 g; ^1H NMR (D_2O) δ 4.85 (dd, J = 33.0, 1.7 Hz, 1 H), 5.56 (dd, J = 46.4, 1.7 Hz, 1 H) [lit.[61] δ 4.88 (dd, J = 33, 2 Hz, 1 H), 5.55 (dd, J = 45, 2 Hz, 1 H)]; ^{13}C NMR (D_2O; reference, MeOH peak at δ 49.0) δ 70.6 (d, J = 20.2 Hz), 89.6 (d, J = 186.8 Hz), 170.3 (d, J = 24.7 Hz), 172.8.

Dimethyl L-*threo*-Fluoromalate. The crude product from the above reaction was dissolved in ether, filtered, and treated with excess diazomethane at 0 °C. The solution was evaporated to a clear oil, which was chromatographed on silica gel [ethyl acetate/hexane (2:3)] to yield crude dimethyl L-*threo*-fluoromalate as a clear colorless oil (1.12 g, 78% from difluorofumarate): TLC [ethyl acetate/hexane (2:3)] R_f 0.23. This material was rechromatographed on silica gel [ether/hexane (1.5:1)] with partial separation of the main product from a less polar minor product, yielding crystalline dimethyl L-*threo*-fluoromalate: 703 mg (49%); mp 53.5-54 °C; IR 3480, 2960, 1745, 1435, 1270, 1215, 1130, 1075, 970, 595 cm^{-1}; $[\alpha]^{22}_D$ -14.1° (c 2.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.25 (d, J = 7.5 Hz, 1 H, OH), 3.84 (s, 3 H), 3.86 (s, 3 H), 4.66 (ddd, J = 30.5, 7.4, 1.7 Hz, 1 H), 5.23 (dd, J = 47.0, 1.7 Hz, 1 H); ^{13}C NMR δ 52.7, 53.2, 71.1 (d, J = 20.8 Hz), 88.7 (d, J = 193.0 Hz), 166.7 (d, J = 24.4 Hz), 170.2. Anal. Calcd for $C_6H_9FO_5$: C, 40.11; H, 5.04. Found: C, 39.87; H, 5.02. A mixed fraction of dimethyl fluoromalates was also obtained, 169 mg (12%).

(+)-MTPA ester of dimethyl L-*threo*-fluoromalate was prepared in the same manner as for chloromalic acids above: ^1H NMR ($CDCl_3$) δ 3.51 (d, J = 0.9 Hz, 3 H), 3.80 (s, 3 H), 3.83 (s, 3 H), 5.45 (dd, J = 46.2, 2.3 Hz, 0.05 H), 5.52 (dd, J = 45.9, 2.3 Hz, 1 H), 5.62 (dd, J = 30.5, 2.3 Hz, 0.05 H), 5.83 (dd, J = 29.7, 2.3 Hz, 1 H), 7.26-7.59 (m, 5 H).

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Registry No. 1, 21788-47-6; 2, 108149-86-6; 3, 108149-87-7; 4, 74134-78-4; 5, 79364-35-5; 6, 79364-36-6; 7, 533-67-5; 8, 108149-88-8; 9, 108149-89-9; 10, 108149-90-2; 11, 108149-91-3; 12, 108149-92-4; 13, 108149-93-5; 14, 108149-94-6; 15, 108149-95-7; 16, 108149-96-8; 17, 105308-75-6; 18, 123-78-4; 19, 2482-37-3; 20, 108149-97-9; 21, 108149-98-0; L-*threo*-fluoromalic acid, 74806-81-8; difluorofumaric acid, 2714-32-1; fumarase, 9032-88-6; dimethyl L-*threo*-fluoromalate, 108149-99-1; dimethyl L-*threo*-fluoromalate ((±)-MTPA ester), 108150-00-1; chlorofumaric acid, 617-42-5.

Isolation and Structure of Bryostatins 10 and 11[1]

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The first two members of a new class of bryostatins designated 10 and 11 have been isolated from Gulf of California and Gulf of Mexico specimens of the marine animal *Bugula neritina* (Bryozoa phylum). Both bryostatins were found to closely resemble bryostatin 4 (**1b**) during isolation (~10^{-7}% yields), and that relationship proved useful in initial structure studies. Definitive structural assignments for bryostatins 10 (**2a**) and 11 (**2d**) were based on analysis of ^{13}C NMR and 400-MHz ^1H NMR spectral data combined with results of selective acetylation (**2a** → **2b**), dehydration, and oxidation (**2a** → **4b** and **2a** → **4d**) experiments. The two new 20-desoxybryostatins 10 (**2a**) and 11 (**2d**) displayed substantial cell growth inhibitory (**2a**, PS ED_{50} 7.6 × 10^{-4} μg/mL, and **2d**, 1.8 × 10^{-5} μg/mL) and antineoplastic activity against the P388 lymphocytic leukemia (PS) with bryostatin 11 leading to a 64% life extension at 92.5 μg/kg. The antineoplastic properties of bryostatins 10 and 11 eliminate an oxygen substituent at C-20 as a prerequisite for such important biological properties.

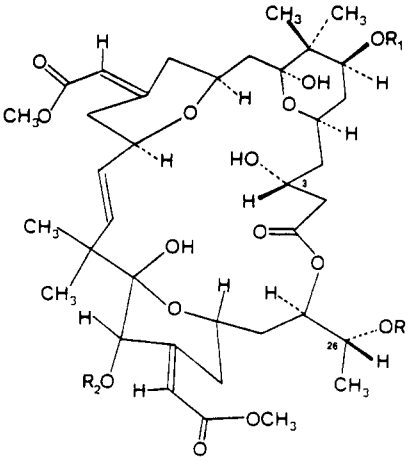
Fossil records suggest many cataclysmic events in evolution of the phylum Bryozoa and a great number of ancient members have become extinct. The 4000 plus species that presently exist represent a very competitive group of animals with highly developed survival mechanisms.[2] Perhaps due to their generally pedestrian appearance and likelihood of being mistaken for seaweeds, hydroids, or corals, these otherwise fascinating "moss animals" have

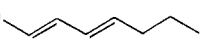
(1) Antineoplastic Agents. 119. For the preceding contribution in this series, see: ref 6f.

(2) For example, refer to: Harvell, C. D. *Science* (Washington D.C.) **1984**, *224*, 1357-1359.

0022-3263/87/1952-2848$01.50/0

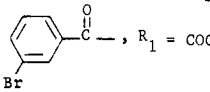
received little biological and chemical study. Because of our observations (in 1968)[3] that extracts of the marine bryozoan *Bugula neritina* (Linnaeus) exhibited exceptional antineoplastic activity (100% life extension) against the U.S. National Cancer Institute murine P388 lymphocytic leukemia (PS system)[4] we undertook an extensive investigation of such constituents and 14 years later reported[5] the isolation and X-ray crystal structure of bryostatin 1 (**1a**) the first member of a very potent (low dose) class of new antitumor substance. Meanwhile we have discovered



1a, R = H, R_1 = $COCH_3$, R_2 = CO  , Bryostatin 1

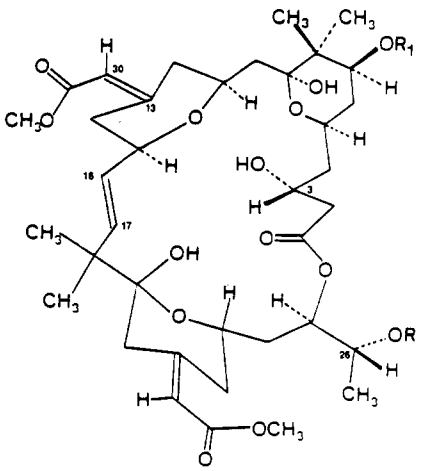
b, R = H, R_1 = $COCH_2CH(CH_3)_2$, R_2 = $COCH_2CH_2CH_3$, Bryostatin 4

c, R = $COCH_3$, R_1 = $COCH_2CH(CH_3)_2$, R_2 = $COCH_2CH_2CH_3$

d, R =  , R_1 = $COCH_2CH(CH_2)$, R_2 = $COCH_2CH_2CH_3$

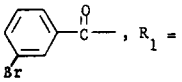
another eight bryostatins[6] of the bryostatins 1 (**1a**) and 4 (**1b**)[6c] types with remarkable (PS inhibition to 10 μg/kg dose levels) antineoplastic activity. In the same period three other bryozoans have been found to contain new pyrrole,[7] indole,[8] quinoline,[9] and purine[10] marine alkaloids. And some of these have displayed antibacterial,[8] antifungal,[10] antialgal,[10] or inhibition of fertilized sea urchin egg cell division[8b] activity.

Continual investigation of biologically active (PS) fractions from Gulf of California and Gulf of Mexico collections of *Bugula neritina* has now led to two new 20-desoxybryostatins herein named 10 and 11 that represent a new class. Both bryostatins 10 (**2a**) and 11 (**2d**)



2a, R = H, R_1 = $COCH_2CH(CH_3)_2$, Bryostatin 10

b, R = $COCH_3$, R_1 = $COCH_2CH(CH_3)_2$

c, R =  , R_1 = $COCH_2CH(CH_3)_2$

d, R = H, R_1 = $COCH_3$, Bryostatin 11

proved to be markedly active against the PS leukemia exhibiting PS cell line growth inhibition at ED_{50} 7.6×10^{-4} and 1.8×10^{-5} μg/mL and in vivo growth inhibition at, for example, 10 μg/kg and 64% at 92.5 μg/kg respectively. The biological activity and physical properties of bryostatins 10 and 11 closely approximated that of bryostatin 4 (**1b**).[6c] While these properties greatly complicated their isolation and purification it did prove useful for simplifying initial characterization. Since bryostatin 10 closely resembles bryostatin 4 and was obtained in somewhat higher yield (7×10^{-7}%) the 33.4 mg obtained from 50 kg (wet weight) of the Gulf of Mexico (Florida) *B. neritina* was subjected to structure determination. Information derived from that study assisted in assigning structure **2d** to the even rarer bryostatin 11 (8.5 mg or 2×10^{-7}% yield) from the same source. The new structures were assigned as follows.

While the optical rotation at $[\alpha]^{27}_D$ +99.8° (methanol) and ultraviolet spectrum with $\lambda_{max}^{CH_3OH}$ 229 nm (ε 36 200) was close to that shown by bryostatin 4, the molecular formula for bryostatin 10 showed a loss by comparison of $C_4H_6O_2$. The molecular formula $C_{42}H_{64}O_{15}$ was derived from the solution-phase secondary ion mass spectrometry (SP-SIMS) procedure[7] that we have developed employing sodium iodide or silver tetrafluoroborate in sulfolane solution. By this means large and easily interpreted molecular ion and fragment ion complexes were formed. The SP-SIMS fragmentation loss of valerate was observed as with bryostatin 4 but not butyrate. Those facts were the first clue to the possibility of a new type of bryostatin bearing a methylene group at C-20. Further evidence for that assumption was obtained by examining the ^{13}C NMR and ^1H NMR spectra obtained from bryostatin 10.

Comparison of the bryostatin 4 and 10 ^{13}C NMR data (cf. **3**) was instructive. The carbon resonance position for carbons 1–19, 22–33, 35–37, and 1′–5′ were almost identical. Most obvious was absence of the butyrate side chain and the upfield shift of C-20 from δ 79.13 in bryostatin 4 to δ 36.17 in bryostatin 10. The adjacent olefin at C-21 → C-34 carbon resonances were shifted from δ 151.88 to 156.98 and

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(7) (a) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.* **1983**, *46*, 917–922. (b) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M. *J. Nat. Prod.* **1984**, *47*, 941–946. (c) Cartē, B.; Faulkner, D. J. *J. Org. Chem.* **1983**, *48*, 2314.
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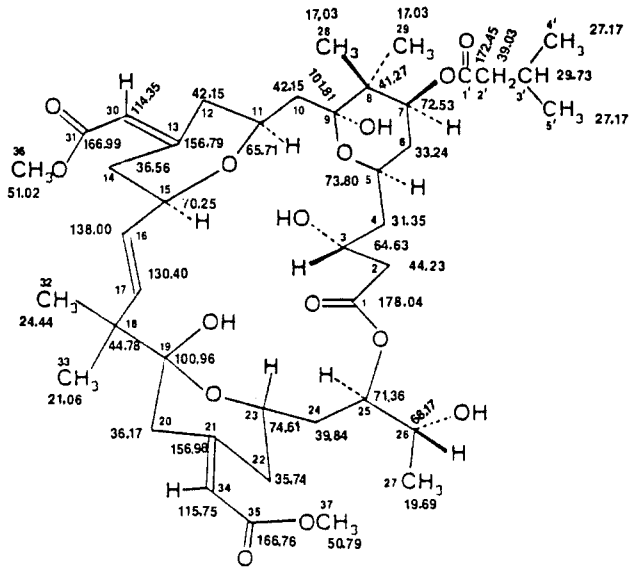


3, Bryostatin 10 (with ^{13}C-NMR)

from 119.68 to 115.75, respectively. Confirmation for the loss of oxygen at C-20 was obtained by comparing the high-field (400 MHz) ^{1}H NMR data derived from bryostatins 4 and 10. The C-20 chemical shift for bryostatin 4 appears at δ 5.167. That signal was absent in bryostatin 10 and replaced by new methylene hydrogens at δ 2.441. Chemical shifts due to protons on the butyrate side chain at C-20 of bryostatin 4 were completely missing in the spectrum of bryostatin 10. Otherwise, the high-resolution ^{1}H NMR spectra of bryostatins 4 and 10 were essentially identical (see Table I). The nearly identical areas of the carbon and proton spectra attributable to positions C-1′ to C-5′ allowed assignment of an isovalerate ester to position C-7.

Given the close relationship of bryostatins 4 and 10, room temperature hydrolysis with 1% hydrochloric acid in methanol was expected to follow an analogous course.[6c] Instead of cleaving the valerate ester as experienced with bryostatin 4, only 1 mol of water was eliminated. The new crystalline compound (0.32 mg) was obtained from 1.2 mg of bryostatin 10 by high-performance liquid chromatography on a C-18 reversed-phase silica gel column using a gradient procedure with methanol–water mixtures. The SP-SIMS spectrum of the degradation product showed a molecular ion complex at m/z 790 (M^+) corresponding to loss of 18 mass units from bryostatin 10. Otherwise, an analogous SP-SIMS fragmentation was found. Also, very helpful was the ultraviolet spectrum which exhibited maxima at 226 (ϵ 305 000) and 301 (ϵ 36 900) nm. The infrared spectra of the dehydration product displayed new absorption at 1660 and 1570 cm^{-1}. Most importantly, the methylene ^{1}H NMR signal at δ 2.441 for the bryostatin 10 C-20 position was missing. Thus, barring an undetected carbonium ion rearrangement the reaction appears to have proceeded by elimination of the C-19 hydroxyl group and formation of a new C-19 → 20 double bond. The resulting diene system and extended chromophore from C-19 → C-35 seemed consistent with the ultraviolet spectrum and added further evidence for assignment of structure **2a** to bryostatin 10. The driving force for dehydration probably resides in the delocalization energy resulting from this extended conjugation system. Interestingly, the dehydration product was formed by allowing bryostatin 10 to remain in methylene chloride–methanol for 1 week at room temperature or for 30 min by adding a drop of 1% hydrochloric acid to a methylene chloride solution of the starting material.

Table I. Bryostatins 10 and 11 ^{1}H NMR (400 MHz) Chemical Shift Assignments (Relative to Tetramethylsilane) in Deuteriochloroform Solution

	bryostatin 10 (**2a**)		bryostatin 11 (**2d**)	
assignmt	δ	mult (J, Hz)	δ	mult (J, Hz)
2	2.45	m	2.449	m
3	4.15	m	4.148	m
4	1.60, 2.05	m, m	1.60, 2.05	m, m
5	4.18	m	4.192	m
6	1.42, 1.74	m, m	1.42, 1.75	m, m
7	5.09	m	5.048	m
10	1.66, 2.16	m, m	1.67, 2.16	m, m
11	3.87	m	3.863	m
12	2.18	m	2.185	m
14	1.85, 2.05	m, m	1.85, 2.05	m, m
15	4.10	m	4.065	m
16	5.421	dd (8.4, 15.8)	5.329	dd (8.46, 15.78)
17	5.799	d (15.8)	5.798	d (15.78)
20	2.441	d (10.5)	2.443	d (10.5)
22	1.85, 2.00	m, m	1.85, 2.00	m, m
23	3.98	m	3.964	m
24	1.78, 1.90	m, m	1.77, 1.95	m, m
25	5.04	m	5.111	m
26	3.74	m	3.807	m
27	1.200	d (6.3)	1.203	d (6.3)
28[a]	1.062	s	1.062	s
29[a]	1.005	s	1.002	s
30	5.663	s	5.667	s
32[a]	1.005	s	0.992	s
33[a]	0.924	s	0.929	s
34	5.677	s	5.676	s
36	3.688	s	3.688	s
37	3.649	s	3.649	s
	C-7 isovalerate		C-7 acetate	
2′	2.23	m	2.027	s
3′	1.85–1.95	m		
4′, 5′	1.17	d (14.5)		

[a] Assignments for these four groups may be interchanged.

To further support the structural assignment **2a** for bryostatin 10 a series of parallel acylation and oxidation experiments were performed with bryostatins 4 and 10. Mild acetylation (1 h at room temperature) of bryostatins 4 and 10 with acetic anhydride–pyridine gave monoacetate derivatives **1c** and **2b**. Introduction of one acetyl group was apparent from appearance of a new ^{1}H NMR sharp singlet at δ 2.056 (for example, see Table II). In both cases the C-26 proton usually seen at δ 3.77 (m) and 3.74 (m) was shifted downfield to δ 4.99 (m) and 5.036 (m), respectively. The ^{13}C NMR spectra verified introduction of one acetyl carbonyl group at δ 170.21 and one methyl group at δ 29.70. Otherwise both the proton and carbon spectra were as expected and almost identical with the parent substances. The SP-SIMS spectrum of bryostatin 10 26-acetate (**2b**) provided further verification with a molecular ion complex corresponding to a mass of 850 and molecular formula $C_{44}H_{66}O_{16}$. Selectivity in the acetylation of bryostatins 4 and 10 at the C-26 hydroxyl group seems consistent with the X-ray crystal structure of bryostatin 1[5] where the C-3 hydroxyl group extends into the macrocyclic lactone cavity and is probably strongly hydrogen bonded. Analogous selectivity was realized when bryostatins 4 and 10 were allowed to react with *m*-bromobenzoyl chloride in pyridine at ice-bath temperature. Again, interpretation of the nuclear magnetic resonance and SP-SIMS spectra established selective esterification of the C-26 hydroxyl group to yield *m*-bromobenzoate esters **1d** and **2c**. Analogous selectivity was again observed when

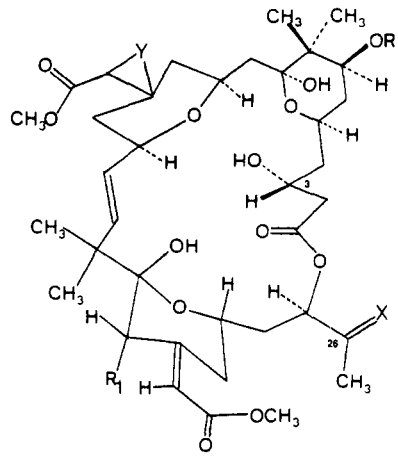
Table II. Bryostatin Acetate ^1H NMR (400 MHz) Chemical Shift Assignments (Relative to Tetramethylsilane) in Deuteriochloroform Solution

	bryostatin 4 acetate (1c)		bryostatin 10 acetate (2b)	
assignmt	δ	mult (J, Hz)	δ	mult (J, Hz)
2	2.43	m	2.462	m
3	4.13	m	4.149	m
4	1.60, 2.03	m, m	1.60, 2.05	m, m
5	4.20	m	4.179	m
6	1.40, 1.71	m, m	1.42, 1.74	m, m
7	5.09	m	5.069	m
10	1.50, 2.05	m	1.66, 2.16	m, m
11	3.80	m	3.849	m
12	2.20	m	2.18	m
14	1.88, 2.10	m, m	1.85, 2.05	m, m
15	4.10	m	4.124	m
16	5.287	dd (8.6, 15.8)	5.323	dd (8.4, 15.7)
17	5.769	d (15.8)	5.811	d (15.7)
20	5.166	s	2.437	d (10.3)
22	1.85, 2.00	m, m	1.85, 2.00	m, m
23	3.97	m	3.919	m
24	1.78, 1.90	m, m	1.78, 1.90	m, m
25	5.27	m	5.294	m
26	4.99	m	5.036	m
27	1.204	d (6.5)	1.243	d (5.9)
28[a]	1.128	s	1.056	s
29[a]	0.990	s	1.004	s
30	5.658	s	5.664	s
32[a]	0.975	s	1.004	s
33[a]	0.921	s	0.927	s
34	5.957	s	5.677	s
36	3.687	s	3.692	s
37	3.653	s	3.650	s
	C-7 Isovalerate			
2′	2.29	m	2.23	m
3′	~1.8–1.9	m	1.85–1.95	m
4′, 5′	1.17	d (14.5)	1.174	d (14.5)
	C-20 Butyrate			
2″	2.29	m		
3″	1.6–1.7	m		
4″	0.912	t (7.2)		
26-$OCOCH_3$	2.056	s	2.055	s

[a] Assignments for these four positions may be interchanged.

bryostatins 4 and 10 were oxidized with chromic acid–pyridine for 1 day at room temperature. The resulting ketones **4a** and **4b** gave analogous SP-SIMS spectra corresponding to introduction of a monoketone and the ^1H NMR spectra showed the C-27 methyl protons as sharp singlets at δ 2.153 and 2.187. The precurser C-26 proton was missing in the spectrum of each ketone.

Completely parallel structural results were also experienced when bryostatins 4 and 10 were allowed to react with *m*-chloroperbenzoic acid in methylene chloride at room temperature. Complete introduction of one oxygen atom took 2 days. The SP-SIMS spectra were entirely consistent with an additional 16 mass units. Instead of epoxidation at the Δ^{16}-olefin of bryostastin 4, the reaction occurred at the $\Delta^{13(30)}$-olefin. That conclusion was reached by detailed interpretation of the ^1H NMR spectra (Table III). The Δ^{16} double bond protons were still present but the C-30 olefin proton that appears at δ 5.658 was shifted to higher field at δ 3.358. In addition, the C-36 methyl ester protons normally at δ 3.687 were slightly shifted to δ 3.774.[6b] As expected, the bryostatin 10 product showed the C-30 proton at δ 3.359 in place of the δ 5.664 for this proton of bryostatin 10 and the 36-methyl group was shifted to δ 3.760. Unequivocal stereochemical assignments for the C-13 → C-30 epoxides and complete elimination



4a, R = $COCH_2CH(CH_3)_4$, R_1 = $OCOCH_2CH_2CH_3$, X = O, Y = π-bond

b, R = $COCH_2CH(CH_3)_4$, R_1 = H, X = O, Y = π-bond

c, R = $COCH_2CH(CH_3)_4$, R = $OCOCH_2CH_2CH_3$, X = H—, HO, Y = O

d, R = $COCH_2CH(CH_3)_4$, R_1 = H, X = H—, HO, Y = O

of epoxidation at the C-21 → C-34 position will require an X-ray crystal structure determination. However, the preceding series of acylation and oxidation experiments combined with detailed analysis of the spectral data clearly allows assignment of structure **2a** to bryostatin 10.

Establishment of bryostatin 10 (**2a**) allowed ready access to the structure of bryostatin 11. The normal phase chromatographic behavior of bryostatin 11 on silica gel was very close to that of bryostatins 4 and 10 and final purification required HPLC procedures on reversed phase silica gel. The SP-SIMS data led to a mass of 766 corresponding to $C_{39}H_{58}O_{15}$ and with obvious loss of an acetyl group indicated that bryostatins 10 and 11 differed by substitution of acetyl for isovalerate. Scrutiny of the 400 MHz ^1H NMR spectrum of bryostatin 11 led to the same conclusion. The proton spectra of bryostatins 10 and 11 were virtually identical except for substitution of an acetyl group methyl signal at δ 2.027 (for the isovalerate ester). The comparison high-field proton spectra have been entered in Table I and firmly support assignment of structure **2d** to bryostatin 11.

The presence of both bryostatins 10 and 11 in specimens of *B. neritina* from the Gulfs of Mexico and California indicates that these new bryostatins will be found in other geographically diverse specimens of this intriguing bryozoan. Since bryostatin 4 also occurs in *B. neritina* from the Gulf of Sagami, Japan,[6c] we anticipate that bryostatins 10 and 11 will eventually be located in this source. Now that the C-20 ester of the bryostatins has been found unnecessary for high antineoplastic activity in the PS leukemia, total synthetic approaches to biologically active bryostatins have been simplified, and our knowledge of structure/activity relationships in this new area has been increased. In summary, discovery of the 20-desoxybryostatins (**2a** and **2d**) provides an important advance toward delineating the path of bryostatin biosynthesis and increases the prospects for uncovering potentially useful anticancer drugs.

Experimental Section

Solvent solutions of reaction mixtures washed with water were dried over anhydrous sodium sulfate. All chromatographic solvents were redistilled. All solvents utilized for extraction, isolation, and reactions were evaporated under reduced pressure with rotary (concentrated or evaporated) evaporators. Commercial sources

Table III. Bryostatin Epoxide ^{1}H NMR (400 MHz) Chemical Shift Assignments (Relative to Tetramethylsilane) in Deuteriochloroform Solution

	bryostatin 4 epoxide (4c)		bryostatin 10 epoxide (4d)	
assignmt	δ	mult (J, Hz)	δ	mult (J, Hz)
2	2.557	m	2.576	m
3	4.168	m	4.155	m
4	1.82, 2.02	m, m	1.80, 2.05	m, m
5	4.255	m	4.257	m
6	1.42, 1.63	m, m	1.42, 1.66	m, m
7	5.103	m	5.104	m
10	1.45, 1.80	m, m	1.48, 1.85	m, m
11	4.125	m	4.120	m
12	2.01	m	2.00	m
14	1.53, 1.72	m, m	1.55, 1.80	m, m
15	4.398	m	4.399	m
16	5.218	dd (8.33, 15.76)	5.220	dd (8.3, 15.8)
17	5.804	d (15.76)	5.824	d (15.8)
20	5.149	s	2.442	d (10.2)
22	1.78, 2.05	m, m	1.78, 2.05	m, m
23	3.998	m	4.002	m
24	1.75, 1.95	m, m	1.75, 1.98	m, m
25	5.163	m	5.160	m
26	3.789	m	3.740	m
27	1.229	d (6.41)	1.210	d (6.3)
28[a]	1.108	s	1.108	s
29[a]	0.9833	s	0.993	s
30	3.358	s	3.359	s
32[a]	0.9586	s	0.984	s
33[a]	0.9018	s	0.903	s
34	5.958	s	5.685	s
36	3.774	s	3.760	s
37	3.659	s	3.652	s
	C-7 Isovalerate			
2′	2.27	m	2.25	m
3′	1.90–2.00	m	1.95–2.00	m
4′, 5′	1.17	d (14.5)	1.17	d (14.5)
	C-20 Butyrate			
2″	2.27	m		
3″	1.60			
4″	0.910	t (7.3)		

[a] Assignments for these four atoms may be interchanged.

of the inorganic (70–230 mesh silica gel) and organic (Sephadex LH-20 gel) employed for column, preparative layer (silica gel), thin-layer (reversed-phase KC-18 or silica gel GF uniplates), and high-performance liquid chromatography (9.4 × 500 mm columns prepared from Partisil-10 M-9 ODS-2 C-18 reversed phase and Partisil-10 M-9) were included in introduction to the Experimental Section of ref. 6c. The thin-layer plates were viewed by ultraviolet light and/or developed with anisaldehyde–acetic acid–sulfuric acid spray with heating to about 150 °C for 5–10 min. Each pure compound was found to be colorless. The instrumental techniques employed for isolation, characterization, and detailed structural elucidation were also summarized in our earlier report.[6c]

Gulf of California *Bugula neritina* (Linnaeus). The 12.5 kg (wet weight) of *B. neritina* (Bryozoa phylum) recollected in 1982 at Bahia Kino-Sonora, Mexico, in January 1982 was identified and initially extracted with 2-propanol followed by 1:1 methylene–chloride–methanol as previously summarized.[6c] The methylene chloride extracts prepared from the 2-propanol preserving solution gave a 50.9-g fraction and water phase gave a 181.9-g fraction. The corresponding methylene chloride and water fractions prepared from the methylene chloride–methanol (1:1) extracts weighed 21.5 and 80.1 g, respectively. Both methylene chloride extracts were significantly active against the P388 lymphocytic leukemia with the former showing T/C 141 → 168 (6.25 → 25 mg/kg) and ED_{50} 3.4 μg/mL and the latter T/C 141 → 170 (6.25 → 25 mg/kg) and ED_{50} 4.8 μg/mL.[8]

Both methylene chloride fractions were combined, dissolved in 9:1 methanol–water, and extracted with hexane. The methanol–water solution was diluted to 4:1 and reextracted with carbon tetrachloride. The resulting hexane (38.4 g), carbon tetrachloride (14.5 g), and 4:1 methanol–water (13.6 g) fractions were subjected to biological evaluation, and the carbon tetrachloride fraction was found to contain the concentrated PS active (T/C 155 → 161 at 3 mg/kg, and ED_{50} 2.0 μg/mL) constituents.

Isolation of Bryostatins 10 (2a) and 11 (2d). A solution of the carbon tetrachloride fraction (14.5 g) prepared from *B. neritina* in methylene chloride was chromatographed on a dry column (3.8 × 100 cm) of silica gel using a gradient of 99:1 to 1:1 methylene chloride–methanol. A 0.291 g fraction with PS T/C 130 → 158 at 200 → 800 μg/kg and ED_{50} 1.1 × 10^{-3} μg/mL was selected for detailed separation guided by PS bioassay and thin-layer chromatography using 90:10:0.8 methylene chloride–methanol–water on silica gel. After being sprayed with the anisaldehyde–acetic acid reagent and heated, the bryostatins appear as a reddish purple color.

The following procedure was found most effective for separating bryostatins 10 and 11 from the closely related bryostatins 4 and 8.[6e] The 0.291-g PS-active fraction was carefully separated by a preparative high-performance liquid chromatographic sequence using first reversed phase with a 1:1 → 9:1 methanol–water gradient followed by normal phase with 150:350:3:0.3 ethyl acetate–heptane–methanol–water. The active fraction (74 mg) containing bryostatins 10 and 11 was obtained as a multicomponent (by TLC with 7:3 hexane–acetone and 4:6 hexane–ethyl acetate) amorphous powder. The new bryostatins were further concentrated by using silica gel column chromatography in a sequence of separations starting with a gradient of 5:1 → 1:1 hexane–acetone and last with a silica gel column utilizing a 3:1 → 1:2 hexane–ethyl acetate gradient. In each case a dry column (1.0 × 60 cm) column technique was performed. Final separation and purification of bryostatins 10 and 11 was realized with reversed-phase HPLC with a 1:1 → 9:1 gradient of methanol–water and a flow rate of 2.0 mL/min. The same procedure was used for final purification of each component and by this means was obtained bryostatin 4 (12.3 mg),[6c] bryostatin 5 (3.0 mg),[6d] bryostatin 6 (2.8 mg),[6d] bryostatin 7 (0.6 mg),[6d] bryostatin 8 (0.5 mg),[6e] bryostatin 10 (9.2 mg, 8 × 10^{-7}% yield),[2a] and bryostatin 11 and 9[6f] as a difficultly separable mixture (2.0 mg). Eventually application of this separation procedure to 50 g (wet weight) of *B. neritina* from the Gulf of Mexico[6c] allowed us to isolate 33.4 mg (7 × 10^{-7}% yield) of bryostatin 10 (**2a**) and 8.1 mg (2 × 10^{-7}% yield) of bryostatin 11 (**2d**) in pure form (see below).

Bryostatin 10. An analytical specimen of bryostatin 10 (**2a**) crystallized from methylene chloride–methanol as plates melting at 161–164 °C: silica gel TLC R_f 0.41 (7:3 hexane–acetone), 0.58 (1:1 hexane–ethyl acetate), and 0.27 (reversed-phase 4:1 methanol–water); MS (SP-SIMS),[7] M^+ 808 $C_{42}H_{64}O_{15}$, with sodium iodide in sulfolane; SP-SIMS, m/z 831.4134 ($[M + Na]^+$, calcd for $C_{42}H_{64}O_{15}Na$ 831.4143), 813 ($[M + Na - 18]^+$), 799 ($[M + Na - 32]^+$), 773 ($[M + Na - 58]^+$), 741 ($[M + Na - 90]^+$), and 729 ($[M + Na - 102]^+$), with silver tetrafluoroborate in sulfolane), 915 and 917 ($[M + Ag^{107}$ and $M + Ag^{109}]^+$), 897 and 899 ([M + Ag isotopes − 18]$^+$), and 879 and 881 ([M + Ag isotopes − 36]$^+$); $[\alpha]^{27}_D$ +99.8° (*c* 0.04, CH_3OH); UV (CH_3OH) λ_{max} 229 (ε 36 200) nm; IR (KBr) 3470, 2980–2945, 1720, 1650, 1645, 1435, 1380, 1370, 1285, 1230, 1150, 1100, 1075, 1060, 1000, and 860 cm^{-1}. The ^{13}C NMR data has been entered on a structure shown previously (see text), and the 400-MHz spectral assignments have been recorded in Table I.

Due to the very valuable nature of bryostatins 4, 10, and 11 and the fact that the mass and nuclear magnetic resonance spectral data provided unequivocal support for the structural assignments, elemental analyses were not pursued.

Bryostatin 11 (2d). A pure sample of bryostatin 11 crystallized from methylene chloride–methanol as needles melting at 171–173 °C: TLC R_f 0.31 (on a reversed-phase plate with 4:1 methanol–water); MS (SP-SIMS),[7] mol wt 766 for $C_{39}H_{58}O_{15}$ from sodium iodide in sulfolane; SP-SIMS, m/z 789.3675 ($M + Na]^+$, calcd for $C_{39}H_{58}O_{15}Na$ 789.3672), 771 ([M + Na − 18]$^+$), 757 ([M + Na − 32]$^+$), 731 ([M + Na − 58]$^+$), 729 ([M + Na − 60]$^+$), and 699 ([M + Na − 90]$^+$); $[\alpha]^{27}_D$ + 42.5° (*c* 0.05, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 227 (ε 35 500) nm; IR (KBr) 3465, 2980–2945, 1740, 1720, 1658–1640, 1440, 1380, 1365, 1280, 1240, 1160, 1095, 1075, 1040, 1000, and 875 cm^{-1}. The quantity of bryostatin 11 was not sufficient for ^{13}C NMR determinations but was very effective for the 400-MHz ^1H NMR spectra summarized in Table I.

Acid-Catalyzed Hydrolysis of Bryostatin 10. Method A. A solution of bryostatin 10 (**2a**, 1.2 mg) in 0.2 mL of methanol containing 1% hydrochloric acid was allowed to remain at room temperature for 3 days. The solvent was concentrated under a stream of nitrogen, and the crude product (1.0 mg) was chromatographed by reversed-phase HPLC on a C-18 column using a gradient of 1:1 to 9:1 methanol–water. The pure $\Delta^{19(20)}$-olefin (0.32 mg) derivative of bryostatin 10 (**2a**) was characterized as described in method C below.

Method B. A solution of bryostatin 10 (**2a**) (0.2 mg) in methylene chloride–methanol (0.2 mL of 4:1) was stored at room temperature for 1 week. By this means 50 μg of the $\Delta^{19(20)}$-olefin was obtained and identified as noted in method C.

Method C. To a solution of bryostatin 10 (**2a**) (1.0 mg) in methylene chloride (0.4 mL) was added one drop of 1% hydrochloric acid in methylene chloride. After 30 min the mixture was poured into ice–water and extracted with methylene chloride. The solvent was washed with water, dried, and concentrated to dryness. Application of the reversed-phase HPLC technique led to 0.41 mg of the $\Delta^{19(20)}$-olefin derivative of bryostatin 10 as plates from methylene chloride–methanol: mp 143–145 °C; TLC (on silica gel) R_f 0.33 (7:3 hexane–acetone) and 0.25 (1:1 hexane–ethyl acetate); MS (SP-SIMS),[7] mol wt 790 for $C_{42}H_{62}O_{14}$ from sodium iodide in sulfolane, which showed m/z 813 ($[M + Na]^+$), 795 ($[M + Na - 18]^+$), 741 ($[M + Na - 72]^+$), 723 ($[M + Na - 90]^+$), and 711 ($[M + Na - 102]^+$); $[\alpha]^{27}_D$ + 96.52° (c 0.04, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 226 (ε 30 500) and 301 (ε 36 900) nm; IR (KBr) 3465, 2980–2940, 1725, 1600, 1650, 1645, 1570, 1440, 1380, 1370, 1285, 1230, 1150, 1100, 1075, 1060, 1000, and 870 cm^{-1}; 400-MHz ^1H NMR (in deuteriochloroform with respect to tetramethylsilane) δ 0.98 and 1.049 (s, C-28 and -29H), 1.17 (d, J = 14.5, side chain dimethyl), 1.211 (d, J = 6.3 Hz, C-27H), 1.232 (s, C-32 and -33H), 2.17 (m, C-12H), 2.45 (m, C-2H), 3.667 (s, C-36), 3.697 (s, C-37H), 5.217 (s, C-20H), 5.412 (dd, J = 8.3 and 15.56 Hz, C-16H), 5.600 (s, C-30H), and 6.010 (d, J = 15.56 Hz, C-17H).

Bryostatin 4 26-Acetate (1c). The acetylation of bryostatin 4 (**1b**, 9.0 mg) was conducted in acetic anhydride (0.4 mL)–pyridine (0.20 mL) at room temperature during 4 h. Upon mixture with ice–water it was extracted with methylene chloride. The chlorocarbon solution was washed with dilute hydrochloric acid and water and dried and the solvent removed. The crude product was purified by HPLC chromatography on a C-18 reversed-phase column (9.4 mm i.d. × 500 mm) using a 1:1 → 9:1 gradient of methanol–water. By this preparative HPLC procedure a pure specimen (8.0 mg) of bryostatin 4 acetate (**1c**) was obtained. Recrystallization from methylene chloride–methanol gave needles melting at 159–162 °C: TLC (on silica gel) R_f 0.49 (7:3 hexane–acetone) and 0.70 (1:1 hexane–ethyl acetate); SP-SIMS,[7] mol wt 936 for $C_{48}H_{72}O_{18}$ from sodium iodide in sulfolane, to give m/z 941 ($[M + Na - 18]^+$), 899 ($[M + Na - 60]^+$), 877 ($[M + Na - 88]^+$), and 857 ($[M + Na - 102]^+$); the high-resolution (400-MHz) ^1H NMR appears in Table II; ^{13}C NMR was consistent with that reported[6c] for bryostatin 4 with addition of the 26-acetate.

In the PS in vitro system bryostatin 4 26-acetate (**1c**) showed ED_{50} 4.5 × 10^{-2} μg/mL.

Bryostatin 10 26-Acetate (2b). A 2.8-mg sample of bryostatin 10 (**2a**) was acetylated and purified as described directly above for bryostatin 4 26-acetate. An analytical specimen crystallized as plates from methylene chloride-methanol and melted at 145–148 °C; TLC (on silica gel) R_f 0.52 (7:3 acetone–hexane) and 0.73 (1:1 hexane–ethyl acetate); SP-SIMS, mol wt 850 for $C_{44}H_{66}O_{16}$ from sodium iodide in sulfolane, where m/z found was 873 ($[M + Na]^+$), 855 ($[M + Na - 18]^+$), 841 ($[M + Na - 32]^+$), 813 ($[M + Na - 60]^+$), 783 ($[M + Na - 90]^+$), and 771 ($[M + Na - 102]^+$); $[\alpha]^{27}_D$ +56.85 (c 0.035, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 228 (ε 36 000) nm; IR (KBr) 3450, 2980–2945, 1740, 1725, 1650, 1645, 1435, 1375, 1360, 1285, 1230, 1150, 1100, 1080, 1060, 1000, and 860 cm^{-1}; ^1H NMR appears in Table II.

Bryostatin 4 26-*m*-Bromobenzoate (1d). To bryostatin 10 (**2a**, 2.5 mg) in pyridine (0.5 mL) was added 0.05 mL of pure *m*-bromobenzoyl chloride at 0 °C. The temperature was maintained at approximately 0 °C for 20 min, and the mixture was diluted with ice–water. After extraction with methylene chloride the solution was washed with water, dilute hydrochloric acid, and water. After removal of the solvent the product was purified by HPLC as described for obtaining bryostatin 4 26-acetate. Recrystallization from methylene chloride–methanol afforded 1.8 mg (72% yield) of the *m*-bromobenzoate **1d** as plates melting at 179–181 °C; MS (SP-SIMS), mol wt 1078 for $C_{53}H_{73}BrO_{18}$, from m/z 1101 ($[M + Na]^+$), 1083 ($[M + Na - 18]^+$), and 1043 ($[M + Na - 58]^+$); $[\alpha]^{27}_D$ +82.7° (c 0.04, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 229 (ε 36 500) and 265 (ε 6700) nm. The IR and proton NMR (at 400 MHz) spectra were consistent with the assigned structure **1d**.

Bryostatin 10 26-*m*-Bromobenzoate (2c). The esterification of bryostatin 10 (**2a**, 2.3 mg) with *m*-bromobenzoyl chloride (0.44 mL) in pyridine (0.50 mL) and isolation of product were conducted as described above for obtaining *m*-bromobenzoate **1d**. A pure sample of 26-*m*-bromobenzoate **2c** (1.84 mg, 80% yield) recrystallized from methylene chloride–methanol as plates: mp 164–168 °C; MS (SP-SIMS), mol wt 991 for $C_{49}H_{67}BrO_{16}$, from m/z 1014 ($[M + Na]^+$), 996 ($[M + Na - 18]^+$), and 956 ($[M + Na - 58]^+$); $[\alpha]^{27}_D$ + 77.4 (c 0.035, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 229 (ε 36 200) and 265 (ε 6800) nm; IR (KBr) 2445, 2980–2950, 1740, 1728, 1658–1640, 1440, 1380, 1365, 1280, 1245, 1160, 1100, 1090, 1085, 1050, 1000, and 875 cm^{-1}. The high-resolution (400-MHz) proton NMR spectrum was as expected for *m*-bromobenzoate **2c**.

26-Oxobryostatin 4 (4a). Chromium trioxide (0.3 mg) was added to a solution of bryostatin 4 (**1b**, 1 mg) in pyridine (0.35 mL). After 20 h at room temperature another 0.1 mg of chromium trioxide was added. Four hours later 2 drops of methanol was added followed by ice–water and extraction with methylene chloride. The combined extract was washed with aqueous sodium bicarbonate solution, dilute hydrochloric acid, and water. Final purification of the product was achieved by HPLC as summarized for the purification of bryostatin 4 26-acetate (see above). An analytical specimen of 26-ketone **4a** (0.52 mg, 52% yield) separated from methylene chloride–methanol as an amorphous powder melting at 213–217 °C: MS (SP-SIMS), mol wt 892 for $C_{46}H_{68}O_{17}$, from m/z 915 ($[M + Na]^+$), 897 ($[M + Na - 18]^+$), 857 ($[M + Na - 58]^+$), 827 ($[M + Na - 88]^+$), and 813 ($[M + Na - 102]^+$); $[\alpha]^{27}_D$ + 88.06 (c 0.034, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 226 (ε 36 200) nm; IR (KBr) 3460, 2980–2930, 1745, 1720, 1645, 1435, 1375, 1360, 1285, 1235, 1165, 1100, 1080, 1025, and 1000 cm^{-1}. The new acetyl group

Table IV. ^1H NMR (400 MHz) Chemical Shift Assignments (Relative to Tetramethylsilane) in Deuteriochloroform Solution for 26-Oxobryostatin 10 (4b)

assignmt	δ	mult (J, Hz)
2	2.539	m
3	4.188	m
4	1.585, 2.097	m, m
5	4.197	m
6	1.419, 1.764	m, m
7	4.903	m
10	1.576, 2.109	m, m
11	3.866	m
12	2.203	m
14	1.877, 2.115	m, m
15	4.055	m
16	5.349	dd (8.33, 15.67)
17	5.694	d (15.67)
20	2.399	d (10.6)
22	1.800, 2.203	m, m
23	4.255	m
24	1.768, 2.139	m, m
25	5.113	m
26		
27[b]	2.187	s
28[a]	1.081	s
29[a]	1.016	s
30	5.662	s
32[a]	1.010	s
33[a]	0.925	s
34	5.707	s
36	3.683	s
37	3.652	s
2′	2.25	m
3′	1.95–2.00	m
4′, 5′	1.17	d (14.5)

[a] Assignments for these four groups may be interchanged. [b] The 27-methyl signal of 26-oxobryostatin 4 appears at 2.153 ppm as a sharp singlet.

methyl appeared at δ 2.153 as a sharp singlet in the ^{1}H NMR spectrum.

26-Oxobryostatin 10 (4b). The oxidation of bryostatin 10 (**2a**) (3.0 mg; with chromium trioxide, 1.2 mg) in pyridine (1.2 mL) and purification of product was executed as summarized for obtaining 26-oxobryostatin 4 (**4a**). Recrystallization from methylene chloride–methanol gave pure plates (1.8 mg, 60% yield) of 26-oxobryostatin 10 (**4b**): mp 163–165 °C; MS (SP-SIMS), mol wt 860 for $C_{42}H_{62}O_{15}$, from m/z 829 ([M + Na]$^+$), 817 ([M + Na − 18]$^+$), 771 ([M + Na − 58]$^+$), 739 ([M + Na − 90]$^+$), 727 ([M + Na − 102]$^+$); $[\alpha]^{27}_D$ + 95.2° (c 0.035, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 227 (ϵ 36 050) nm; IR (KBr) 3450, 2980–2938, 1745, 1724, 1650, 1435, 1370, 1360, 1285, 1240, 1165, 1100, 1085, 1045, 1025, 1000, 870 cm^{-1}. The high-resolution proton NMR spectrum and interpretation has been summarized in Table IV.

13,30-Epoxybryostatin 4 (4c). To a solution of bryostatin 4 (**1b**, 3.2 mg) in methylene chloride (0.5 mL) was added 1.5 mg of *m*-chloroperbenzoic acid, and the mixture was allowed to remain at room temperature for 48 h. Upon dilution with ice–water and extraction with methylene chloride the solution was washed with aqueous sodium bisulfite and potassium iodide followed by water. After removal of the solvent, the crude product (3.1 mg) was purified by the same HPLC method described above for obtaining acetate **1c**. Recrystallization from methylene chloride–methanol afforded 2.0 mg (62.5% yield) of the 13,30-epoxide **4c** as plates melting at 189–192 °C: MS (SP-SIMS), mol wt 910 for $C_{46}H_{70}O_{18}$, from m/z 933 ([M + Na]$^+$), 915 ([M + Na − 18]$^+$), 901 ([M + Na − 32]$^+$), 875 ([M + Na − 58]$^+$), 861 ([M + Na − 72]$^+$), 845 ([M + Na − 88]$^+$), and 831 ([M + Na − 102]$^+$); $[\alpha]^{27}_D$ + 56.75 (c 0.04, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ 227 (ϵ 35 800) 227 nm; IR (KBr) 3450, 2985–2440, 1740, 1725, 1660–1640, 1440, 1380, 1360, 1290, 1240, 1160, 1100, 1080, 1060, 1045, 1000, and 870 cm^{-1}. The proton NMR data is given in Table III.

13,30-Epoxybryostatin 10 (4d). The epoxidation of bryostatin 10 (**2a**, 3.0 mg) was pursued and epoxide **4d** isolated in 63% yield (1.9 mg) as summarized above in the parallel experiment employing bryostatin 4. After recrystallization from methylene chloride–methanol the 13,30-epoxide **4d** was obtained as plates melting at 184–186 °C; MS (SP-SIMS), mol wt 824 for $C_{42}H_{64}O_{16}$, from m/z 847 ([M + Na]$^+$), 829 ([M + Na − 18]$^+$), 815 ([M + Na − 32]$^+$), 789 ([M + Na − 58]$^+$), 757 ([M + Na − 90]$^+$), and 745 ([M + Na − 102]$^+$); $[\alpha]^{27}_D$ +88.5° (c 0.035, CH_3OH); UV λ_{max} 226 (ϵ 36 000) nm; IR (KBr) 3460, 2980–2940, 1740, 1720, 1650, 1435, 1380, 1358, 1283, 1235, 1165, 1100, 1080, 1030, 1000, and 872 cm^{-1}. A summary of the proton NMR spectrum with assignments is given in Table III.

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Isolation and Structure of Bryostatins 12 and 13[1a]

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Bryostatins 12 (**4**, 3.7 mg) and 13 (**5**, 0.7 mg) were isolated from ~1000 kg of the marine bryozoan *Bugula neritina* (Linnaeus) found in the Eastern Pacific Ocean (California). The new bryostatins led to strong cell growth inhibitory (PS cell line ED_{50} 0.014 and 0.0054 μg/mL) and antineoplastic activity (for bryostatin 12, a 47–68% increase in life extension at 30–50 μg/kg) against the marine P388 lymphocytic leukemia (PS system). In addition to the previously known constituents (bryostatins 1–3) of *Bugula neritina* from this ocean area the presence of bryostatins 8 (13.2 mg) and 9 (16.4 mg) was also established. A detailed series of ^{1}H–^{1}H COSY, 2D-*J*-resolved, ^{1}H–^{13}C 2D-shift correlated, and ^{1}H–[^{1}H] NOE difference ^{1}H NMR experiments combined with ^{13}C NMR and SP-SIMS studies were employed to elucidate the structures of bryostatins 12 and 13.

Chemical and biological evaluation of Bryozoan biosynthetic products appears to offer exceptional scientific and medical potential. The seven species[2,3] (of 4000+) of these fascinating marine animals so far examined have afforded marine alkaloids[4] and macrocyclic lactones[2,5] as diverse as the remarkable β-lactam chartelline A[3] from *Chartella papyracea* and bryostatin 1 (**1a**)[6] from *Bugula neritina*. Bryostatin 1 (**1a**) has been found to provide a 96% increase in life-span against the U.S. National Cancer Institute's P388 lymphocytic leukemia (PS system) at 70 μg/kg with activity starting at very low (<10 μg/kg) dose levels. Presently bryostatin 1 is undergoing preclinical

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