87870-13-1; **17a** 6,7-dihydro picrate, 87870-15-3; **17b**, 87870-16-4; **17c**·HCl 6,7-dihydro, 87870-17-5; **17d**·HCl 6,7-dihydro, 87870-18-6; **18**·HCl 4,5-dihydro, 87870-19-7; (*E*)-**21a**, 83575-90-0; (*Z*,*E*)-**21b**, 87870-20-0; **22a**, 87870-21-1; **22b**, 87870-22-2; **23**, 87870-23-3; (*Z*,*E*)-**24a**, 87870-24-4; (*Z*,*E*)-**24b**, 87870-25-5; **25a**, 87870-26-6; **25b** 1,2-dihydro dipicrate, 87870-28-8; **26a** 5,6-dihydro dipicrate, 87870-30-2; (*Z*,*E*)-**27a**, 87870-31-3; (*Z*,*E*)-**27b**, 87870-32-4; **28**·HCl 1,9a-dihydro, 87870-33-5; **29**, 87870-34-6; (*Z*,*E*)-**30**, 87870-35-7; **31** 1,2-dihydro picrate, 87870-37-9; (*E*,*E*)-**33**, 87870-38-0; (*E*,*Z*)-**33**, 87870-39-1; (*Z*,*E*)-**33**, 87870-40-4; (*Z*,*Z*)-**33**, 87870-41-5; **34**·HCl 1,2-dihydro, 87870-42-6; acetophenone, 98-86-2; 2-propenylamine, 107-11-9; 4-benzoylpyridine, 14548-46-0.

Notes

Structure of the *Bugula neritina* (Marine Bryozoa) Antineoplastic Component Bryostatin 3[1]

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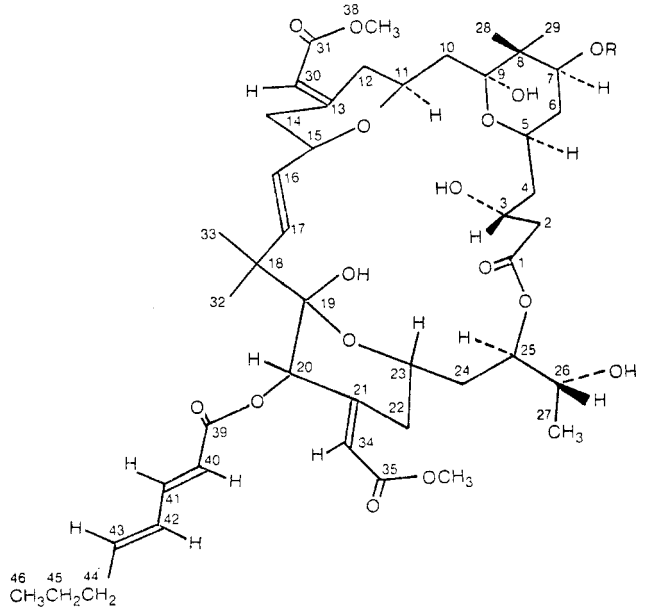
The marine Bryozoan *Bugula neritina* (Linnaeus) was formally described in 1758 and is now recognized as a common fouling organism on marine facilities and equipment. Colonies of this very cosmopolitan species may reach a height of 10 cm, where each tiny animal unit ranges from a width of 0.2–0.3 mm to a length of 0.6–0.8 mm.[2] In preceding reports[3] we summarized the discovery of 17 exceptionally potent *B. neritina* antineoplastic constituents and structures for the first two members of the series: bryostatins 1 (**1a**)[3a] and 2 (**1b**).[3b] These extraordinary

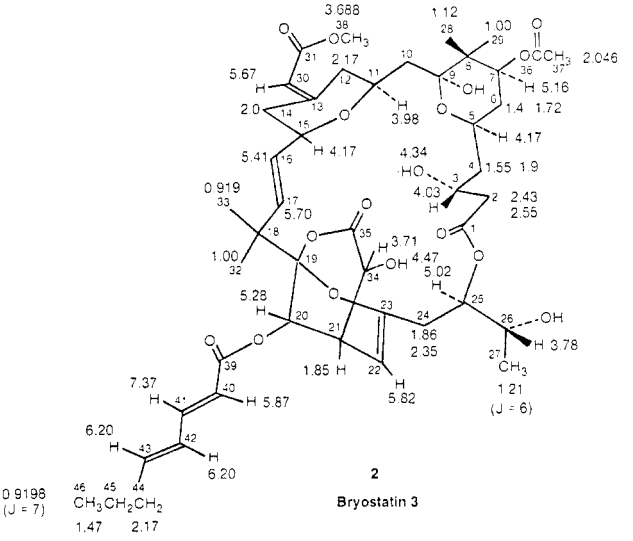


1a, R = $CO\text{-}CH_3$

b, R = H

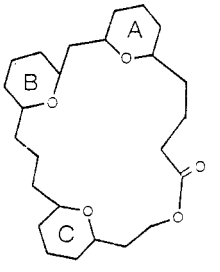
20-membered-ring lactones suggest that an intriguing series of biochemical events may be responsible for their powerful antineoplastic activity. Indeed, the possibility of affecting cellular membranes with such cyclic ionophores[4] suggests the added prospect of tumor destruction at the cellular level.[5] In pursuit of such important questions and the prospect of further defining structure/activity relationships, we have studied another novel *B. neritina* antineoplastic component herein designated bryostatin 3.

We now report the bioassay (PS system) guided isolation (81.5 mg, 1.6×10^{-7} % yield) and structural elucidation of bryostatin 3 (**2**) from *Bugula neritina*. Isolation of crude



2

Bryostatin 3



3

Bryopyran

bryostatin 3 was performed by employing the general route summarized for obtaining bryostatin 1.[3a] Bryostatin 3 (**2**) was found to strongly inhibit (life extension of 63% at 30

(1) Antineoplastic Agents. 93. For part 92 refer to: Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.*, in press.

(2) Morris, R. H.; Abbott, D. P.; Haderlie, E. C. "Intertidal Invertebrates of California"; Stanford University Press: Stanford, CA, 1980; p 96.

(3) (a) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846. (b) Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R. *J. Nat. Prod.* **1983**, *46*, 528.

(4) We have observed bryostatins 1–3 to complex strongly with silver ion in FAB mass spectrometry experiments (see ref 1). Alternatively, this might only be due to the diene side chain at C-20.

(5) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305.

0022-3263/83/1948-5354$01.50/0

μg/kg) growth of the U.S. National Cancer Institute's (NCI) P388 lymphocytic leukemia (PS system). A chromatographically pure specimen of this sensitive biosynthetic product was obtained as an amorphous solid. Attempts at crystallization resulted in decomposition.

Analysis of the FAB mass spectra of bryostatin 1 (**1a**) and 3 (**2**) indicated a net loss of 16 mass units for bryostatin 3. Furthermore, the FAB fragmentation pattern involving loss of the acetate and octa-2,4-dienoate side chains of bryostatin 1 was repeated in the spectrum of bryostatin 3. The similarity of optical rotations and ultraviolet spectra exhibited by bryostatins 1–3 also suggested that the new macrocyclic lactone (**2**) resembled its companions. The ^{13}C NMR spectra led to the same overall conclusion and indicated that bryostatins 1–3 contained the same bryopyran (**3**) ring system. However, some signal shifts (1–3 ppm) and other differences were noted. Signals previously[3b] assigned to carbons C-21 and C-47 of bryostatins 1 and 2 were missing, and that due to C-35 appeared to undergo a downfield shift from 166.8 to 171.9 ppm. The ^{13}C NMR spectrum of lactone **2** also contained new signals at 41.82, 68.30, 114.12, and 166.73 ppm, now believed to correspond to C-21, C-34, C-22, and C-23, respectively.

Careful interpretation of the infrared and high-resolution (400 MHz) ^1H NMR spectra (decoupling experiments) allowed a definite structural assignment (**2**) for bryostatin 3. The new infrared absorption band at 1785 cm^{-1} exhibited by bryopyran 2 indicated a possible five- or six-membered lactone carbonyl group with an α electronegative substituent. Comparison of the ^1H NMR spectra showed loss of the C-22 and C-23 protons of bryostatins 1 and 2. And protons assigned to C-15–17, C-20, C-24, and C-40–43 were shifted downfield from those of bryostatin 1 by 0.01–0.05 ppm. An upfield shift of about the same magnitude was viewed for the C-17 and C-25 protons. The C-24 proton was strongly displaced downfield from δ 2.0 to 2.35. Very importantly, new signals appeared in the spectrum of bryostatin 3 at δ 5.82 (C-22) and at δ 3.71 (C-34). The latter was coupled to protons at δ 1.85 (C-21) and at δ 4.47 (OH at C-34). From these NMR interpretations it was clear that the fundamental bryopyran ring system (**3**) was still represented in bryostatin 3. Indeed, except for a profound change in the bryopyran C-ring and the shifts noted above, the remainder of bryostatin 3 was found essentially identical with bryostatin 1 (**1a**). A shift (C-21,34 → C-22,23) in the exocyclic ring C olefin of bryostatin 1 to a dihydropyran system, hydroxylation at C-34, and lactonization of the C-35 carbonyl of the lactate side chain with the C-19 hemiketal hydroxyl group provided a most satisfactory explanation of the bryostatin 3 to bryostatin 1 relationship. The preceding observations combined with the unequivocal X-ray crystal structure determination[3a] of bryostatin 1 (**1a**) allowed structure **2** to be assigned to bryostatin 3.

Since bryostatin 3 (**2**) retains the very potent antineoplastic activity of bryostatin 1 (**1a**),[6] loss of one pyran acetylidene group does not appear critical. Indeed, present evidence suggests that substantial structural modifications can be made in the bryostatin ester substituents while preserving antineoplastic activity. In addition to the obvious potential of the bryostatins for eventually contributing to improvements in cancer treatment as the original biosynthetic product or through structural modifications, a number of other stimulating biochemical questions are posed by discovery of these unique and biologically potent new macrocyclic lactones. For example, are the bryostatins endogenous, or are they derived from common bryozoan food sources such as bacteria and phytoplankton, and do they serve as defensive substances? Aside from some fish, *Bugula neritina* has only one known predator: *Polycera atra*.[2] If the bryostatins are in turn utilized for defensive purposes by this animal (Mollusca phylum), its common description as the sorcerer's nudibranch will prove to be exceedingly apt.

Experimental Section

Column chromatography was performed by using either Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) or silica gel 60 (E. Merck, Darmstadt; prepack columns, sizes A and B). Fractions were collected with either Gilson FC-80 or FC-220 equipment. Mass spectra were recorded with a MAT-312 mass spectrometer and infrared spectra with a Nicolet FTIR Model MX-1 spectrometer. The NMR spectra were obtained by using Bruker WH-90 and WH-400 instruments. Chemical shifts are reported as δ values relative to tetramethylsilane as an internal standard in deuteriochloroform.

Collection and Extraction. *Bugula neritina* (500 kg wet weight) was collected in the Eastern Pacific Ocean (California). The marine animal was preserved in 2-propanol. The 2-propanol solution (577 L) was decanted and concentrated to an aqueous slurry which was extracted with methylene chloride. The initial methylene chloride extract was separated by the solvent partition sequence 9:1 to 4:1 methanol–water with ligroin–carbon tetrachloride.[7]

The carbon tetrachloride fraction (214 g) in 1:1 methylene chloride/methanol was first separated by using gel permeation chromatography on Sephadex LH-20.

Bryostatin 3. A PS-active fraction (168.5 mg) from the Sephadex LH-20 separation used as a source of bryostatin 2, **3b** was further separated on a column of silica gel (three Merck size B in series) by using a solvent gradient of methylene chloride to 85:5 methylene chloride/methanol to give a total of 287 fractions (5 mL each). Bryostatin 3 was contained in fractions 130–5 (72.2 mg). Further purification by using the same gradient and three Merck size A columns in series led to bryostatin 3 which was eluted by the gradient 99:1 to 97:3 methylene chloride/methanol. A total of 144 fractions were collected, and bryostatin 3 was found in fractions 92–8: 42.3 mg; $[\alpha]^{25}_D$ +61° (*c* 0.26, methanol); UV (CH_3OH) λ_{max} 230 nm (ϵ 36 000), and 261 (35 000); FAB mass spectrum, *m/e* 911 (M + Na where M = $C_{46}H_{64}O_{17}$) and the base peak, 851 (M + Na − 60), 787 (M + Na − 124), 771 (M + Na − 140); IR (KBr), 3450, 2930–2970, 1785, 1740, 1718, 1640–1650, 1365, 1305, 1250, 1165, 1150, 1135, 1100, 1045–1075, 1000 cm^{-1}; HR (400 MHz) ^1H NMR (in deuteriochloroform; see structure **2**); ^{13}C NMR δ 172.48, 171.96, 171.18, 167.12, 166.89, 165.98, 157.11, 147.72, 146.65, 136.38, 132.77, 128.38, 117.37, 114.38, 114.12, 101.90, 101.71, 81.24, 73.05, 73.05, 71.46, 69.77, 69.12, 68.60, 68.30, 65.58, 51.18, 45.23, 44.19, 41.82, 41.82, 41.14, 39.48, 36.43, 35.13, 35.13, 33.24, 33.24, 33.24, 24.50, 21.87, 21.15, 21.15, 19.30, 16.99, 13.68.

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(6) So far bryostatin 1 has been shown in studies conducted by the NCI to display very potent (low dose) activity against, e.g., the murine PS (52–96% life extension at 10–70 μg/kg), L1210 (lymphocytic leukemia, 34–51% life extension at 37.5–150 μg/kg), and M5 (M5076 ovarian carcinoma, 40–48% life extension at 5–20 μg/kg and 20–65% curative in the tumor regression model at 20–40 μg/kg) key experimental tumor systems.

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Registry No. Bryostatin 3, 87370-86-3.

A Direct Synthesis of β-Hydroxybutyrolactones: Total Synthesis of Dendrolasin and Formal Total Synthesis of Aplysistatin

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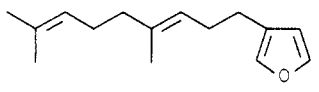
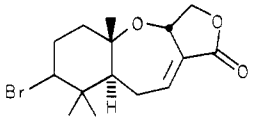
The β-hydroxybutyrolactone unit has been employed as a building block for the synthesis of several furans and sesquiterpenes. Negishi and co-workers[1] developed a clever route to dendrolasin (1). Recently Prestwich and Shieh[2]



1



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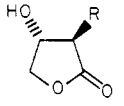
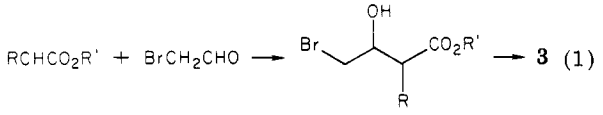


3

3a, R = $(CH_3)_2C{=}CH(CH_2)_2C(CH_3){=}CH(CH_2)_2$-

reported an elegant synthesis of aplysistatin (2) that used **3a** as a key intermediate. The utility of **3** has been enhanced by a study of the alkylation chemistry of its dianion[3] and by advances in the synthesis of **3**.[4] We recently developed a convenient synthesis of anhydrous bromoacetaldehyde[5] and now report the use of this reagent for the synthesis of several β-hydroxybutenolides. The overall plan is depicted in eq 1.

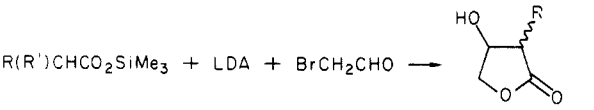


$$RCHCO_2R' + BrCH_2CHO \longrightarrow \text{4} \longrightarrow \mathbf{3} \quad (1)$$

4

While the anions of the ethyl esters of aliphatic acids[6] reacted rapidly with bromoacetaldehyde to produce **4** (R′ = Et), the hydrolysis resulted in complex product mixtures. Presumably competitive formation of an epoxy ester and its subsequent base-mediated transformations were responsible. The reaction of carboxylic acid dianions[7] with bromoacetaldehyde failed due to the polymerization of the bromoacetaldehyde. Although the *tert*-butyl ester anions afforded the β-hydroxy esters **4** in good yields, subsequent attempts to remove the *tert*-butyl group (pTSA, PhH ↑↓; Me_3SiI, CH_2Cl_2) resulted in only modest yields of the desired bromo hydroxy acid. In contrast, the trimethylsilyl ester anions[6] afforded a mixture of **3** and **5** (eq 2). The

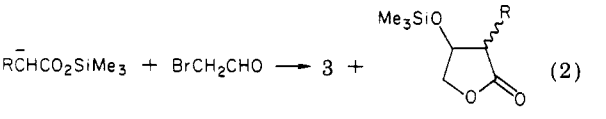
Table I. Synthesis of β-Hydroxybutyrolactones



$$R(R')CHCO_2SiMe_3 + LDA + BrCH_2CHO \longrightarrow$$

entry	R	R′	yield, %
1	CH_3	CH_3	61
2	*n*-Bu	H	90
3	$H_2C{=}CHCH_2$	H	62[b]
4	C_6H_5O	H	80
5	C_6H_5S	H	65[a]
6	C_6H_5Se	CH_3	75
7	Br	Br	
8	$H(CH_2C(CH_3){=}CHCH_2)_2CH_2$	H	99

[a] A 30% yield of the corresponding butenolide was also obtained. [b] Entry 3 was previously prepared in ref 3b.

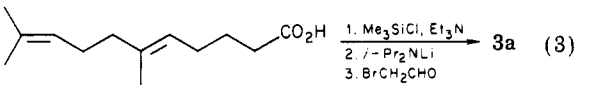


$$R\bar{C}HCO_2SiMe_3 + BrCH_2CHO \longrightarrow \mathbf{3} + \text{5} \quad (2)$$

5

complete conversion to **3** could be effected by quenching the reaction with tetra-*n*-butylammonium fluoride. The results are illustrated in Table I.

Most silyl esters were converted to **3** in good to excellent yields. However, if the substituent R was one that increased the acidity of the α-proton (entry 5), varying amounts of the corresponding butenolide were obtained. The hydroxybutenolides were initially produced as an equal mixture of diastereomers but could be converted to the trans isomer.[9] The synthesis of the key intermediate in the Prestwich synthesis of aplysistatin is shown in eq 3. Acid **6**[8] was converted to its trimethylsilyl ester and



$$\text{6} \xrightarrow[2.\ i\text{-}Pr_2NLi\ \ 3.\ BrCH_2CHO]{1.\ Me_3SiCl,\ Et_3N} \mathbf{3a} \quad (3)$$

6

was reacted sequentially with lithium diisopropylamide (LDA) and bromoacetaldehyde to provide **3a** as an equal mixture of diastereomers in 99% yield. One of the diastereomers was identical with authentic sample. The hydroxy lactone **3a** was dehydrated to the butenolide and then reduced with diisobutylaluminum hydride.[10] Acidic workup of the reduction furnished dendrolasin (1) in 32% yield from **3a** (eq 4).

$$\mathbf{3a} \xrightarrow[2Et_3N]{CH_3SO_2Cl} \xrightarrow[\text{then } H^+]{(i\text{-}Bu)_2AlH} \mathbf{1} \quad (4)$$

Since higher molecular weight α-bromoaldehydes are readily available, the approach described herein should permit the synthesis of several butenolides, hydroxybutyrolactones, and tetronic acids. The synthesis of **3a** constitutes a formal total synthesis of aplysistatin.

Experimental Section

General Experimental Procedure for Synthesis of 3. To a dry flask containing a nitrogen atmosphere was added 1 mL of dry THF (distilled from $LiAlH_4$) and 0.15 mL of diiso-

(1) Kobayashi, M.; Negishi, E. *J. Org. Chem.* **1980**, *45*, 5225.
(2) Shieh, H. M.; Prestwich, G. D. *Tetrahedron Lett.* **1982**, *23*, 4643.
(3) (a) Chamberlin, A. R.; Dezube, M. *Tetrahedron Lett.* **1982**, *23*, 3055. (b) Shieh, H. M.; Prestwich, G. *J. Org. Chem.* **1981**, *46*, 4319.
(4) Mukaiyama, T.; Fujimoto, K.; Hirose, T.; Takeda, T. *Chem. Lett.* **1980**, 635. Takeda, A. *Tetrahedron Lett.* **1982**, *23*, 5185.
(5) Kraus, G. A.; Gottschalk, P. *J. Org. Chem.* **1983**, *48*, 2111.
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(7) Pfeffer, P. E.; Silbert, L. S. *J. Org. Chem.* **1972**, *37*, 451, 1256.
(8) Grieco, P. A. *J. Am. Chem. Soc.* **1969**, *91*, 5660.
(9) The conversion was effected by stirring with potassium *tert*-butoxide in THF at room temperature.
(10) Minato, H.; Nagasaki, T. *J. Chem. Soc. C* **1968**, 621.

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