1h (97%): deep green prisms (hexane); mp 117–119 °C; IR 3050, 2950, 1590, 1455, 1340, 1225, 1110, 1060, 970, 930, 880, 790, 660 cm^{-1}; ^1H NMR δ −3.97 (3 H, s), −3.92 (3 H, s), 1.59 (3 H, t, J = 7 Hz), 1.68 (9 H, s), 1.69 (9 H, s), 3.64 (2 H, q, J = 7 Hz), 8.4–8.7 (7 H, m); EIMS m/e 372 (M^+). Anal. Calcd for $C_{28}H_{36}$: C, 90.26; H, 9.74. Found: C, 90.01; H, 9.82.

1i (96%): deep green prisms (ethanol); mp 129–130 °C; IR 3050, 2980, 2950, 2880, 1600, 1470, 1440, 1380, 1360, 1340, 1230, 1120, 880 cm^{-1}; ^1H NMR δ −3.99 (3 H, s), −3.94 (3 H, s), 1.07 (3 H, t, J = 7 Hz), 1.68 (9 H, s), 1.69 (9 H, s), 2.05 (2 H, tq, J = 8 Hz, J = 7 Hz), 3.58 (2 H, t, J = 8 Hz), 8.36–8.48 (6 H, m), 8.70 (1 H, s); EIMS m/e 386 (M^+). Anal. Calcd for $C_{29}H_{38}$: C, 90.09; H, 9.91. Found: C, 90.34; H, 9.85.

2,7-Di-*tert*-butyl-*trans*-10b,10c-dimethyl-10b,10c-dihydropyrene-4-aldoxime (1j). To a solution of 420 mg (1.09 mmol) of **1b** in 40 mL of ethanol was added 2.0 g (28.6 mmol) of hydroxylamine hydrochloride. The pH of the solution was adjusted to pH 7 with 10% aqueous Na_2CO_3. After the mixture was warmed on steam bath for 1 h, it was cooled to room temperature. A green precipitate was removed by filtration. This solid was recrystallized from ethanol to give 417 mg (95%) of **1j**: green prisms; mp 214–216 °C; IR 3350, 3050, 2950, 2350, 1900, 1500, 1450, 1360, 1225, 930, 880, 660 cm^{-1}; ^1H NMR δ −3.78 (3 H, s), −3.94 (3 H, s), 1.57 (9 H, s), 1.67 (9 H, s), 5.30 (1 H, s), 8.50 (4 H, m), 8.52 (1 H, s), 8.81 (1 H, s), 9.47 (1 H, s); EIMS m/e 387 (M^+). Anal. Calcd for $C_{27}H_{33}NO$: C, 83.68; H, 8.58; N, 3.61. Found: C, 83.14; H, 8.47; N, 3.59.

2,7-Di-*tert*-butyl-4-cyano-*trans*-10b,10c-dimethyl-10b,10c-dihydropyrene (1k). A solution of 301 mg (1.16 mmol) of **1j** in 20 mL of acetic anhydride was refluxed for 3 h. After cooling to room temperature, the mixture was poured into water. When the reaction of excess acetic anhydride with water was completed, the mixture was extracted with 30 mL of dichloromethane. The extract was washed with saturated aqueous NaCl and then was concentrated. The residue was recrystallized from methanol to give 283 mg (87%) of **1k**: green prisms (methanol); mp 212–213 °C; IR 3020, 2950, 2200, 1590, 1455, 1380, 1345, 1230, 1150, 1065, 930, 880, 790, 680, 670 cm^{-1}; ^1H NMR δ −3.96 (3 H, s), −3.95 (3 H, s), 1.69 (9 H, s), 1.73 (9 H, s), 8.5–8.7 (6 H, m), 8.91 (1 H, s); EIMS m/e 369 (M^+). Anal. Calcd for $C_{27}H_{31}N$: C, 87.75; H, 8.46; N, 3.79. Found: C, 87.47; H, 8.39; N, 3.89.

General Procedure for Reaction of 1 with Iodine. A solution of 400 mg (1.16 mmol) of **1a** and 1220 mg (4.8 mmol, 4.2 equiv) of iodine in 30 mL of toluene was refluxed for 48 h. The cooled mixture was washed with 10% aqueous sodium thiosulfate and water. The organic layer was then evaporated. The residue was dissolved in a small amount of benzene and was chromatographed on silica gel (hexane) to give 360 mg (97%) of **2a** as a colorless solid, which was recrystallized from ethanol. **2a**: colorless prisms (ethanol); mp 209–210 °C (lit.[12] mp 208–209 °C). Compounds **2b**, **2c**, **2g**, **2h**, and **2i** were prepared in a similar manner.

2b (60%): yellow prisms (hexane); mp 175–177 °C; IR 3050, 2950, 2700, 1675, 1590, 1450, 1380, 1350, 1200, 1165, 890, 710 cm^{-1}; ^1H NMR δ 1.59 (9 H, s), 1.61 (9 H, s), 8.03 (1 H, d, J = 6 Hz), 8.07 (1 H, d, J = 6 Hz), 8.27 (1 H, d, J = 1.6 Hz), 8.34 (1 H, d, J = 1.4 Hz), 8.36 (1 H, d, J = 1.4 Hz), 8.59 (1 H, s), 9.73 (1 H, d, J = 1.6 Hz), 10.54 (1 H, s); EIMS m/e 342 (M^+). Anal. Calcd $C_{25}H_{26}O$: C, 87.67; H, 7.65. Found: C, 87.58; H, 7.64.

2c (72%): brown yellow prisms (chloroform); mp 121–122 °C; IR 3000, 2940, 1680, 1610, 1490, 1470, 1400, 1370, 1275, 1235, 1210, 890, 730, 695 cm^{-1}; ^1H NMR δ 1.58 (18 H, s), reduced, 2.93 (3 H, s), 7.99 (1 H, d, J = 9 Hz), 8.04 (1 H, d, J = 9 Hz), 8.25 (1 H, d, J = 2 Hz), 8.27 (1 H, s), 8.59 (2 H, s), 9.23 (1 H, d, J = 2 Hz); EIMS m/e 356 (M^+). Anal. Calcd for $C_{26}H_{28}O$: C, 87.59; H, 7.92. Found: C, 87.51; H, 7.97.

2g (87%): colorless prisms (ethanol); mp 172–175 °C; IR 3050, 2950, 1600, 1470, 1450, 1360, 1220, 880, 790, 715 cm^{-1}; ^1H NMR δ 1.56 (9 H, s), 1.59 (9 H, s), 2.89 (3 H, s), 7.88 (1 H, s), 8.00 (2 H, s), 8.12 (2 H, s), 8.19 (1 H, d, J = 1.8 Hz), 8.29 (1 H, d, J = 1.8 Hz); EIMS m/e 328 (M^+). Anal. Calcd for $C_{25}H_{28}$: C, 91.41; H, 8.59. Found: C, 91.70; H, 8.68.

2h (73%): colorless prisms (methanol); mp 110–113 °C; IR 3020, 2920, 2850, 1590, 1445, 1385, 1350, 1260, 1220, 1195, 870, 710 cm^{-1}; ^1H NMR δ 1.56 (3 H, t, J = 7.0 Hz), 1.57 (9 H, s), 1.59 (9 H, s), 3.32 (2 H, q, J = 7.0 Hz), 7.90 (1 H, s), 8.01 (2 H, s), 8.12 (1 H, d, J = 2.0 Hz), 8.14 (1 H, d, J = 2.0 Hz), 8.18 (1 H, d, J = 2.0 Hz), 8.38 (1 H, d, J = 2.0 Hz); EIMS m/e 342 (M^+). Anal. Calcd for $C_{26}H_{30}$: C, 91.17; H, 8.83. Found: C, 91.04; H, 8.89.

2i (75%): colorless prisms (methanol); mp 87–90 °C; IR 3020, 2900, 2850, 1590, 1445, 1380, 1350, 1265, 1220, 1190, 870, 710 cm^{-1}; ^1H NMR δ 1.13 (3 H, t, J = 7.3 Hz), 1.57 (9 H, s), 1.59 (9 H, s), 1.98 (2 H, tq, J = 6.5, 7.3 Hz), 3.26 (2 H, t, J = 6.5 Hz), 7.88 (1 H, s), 8.00 (2 H, s), 8.13 (2 H, s), 8.17 (1 H, d, J = 1.6 Hz), 8.36 (1 H, d, J = 1.6 Hz); EIMS m/e 356 (M^+). Anal. Calcd for $C_{27}H_{32}$: C, 90.95; H, 9.05. Found: C, 90.83; H, 9.00.

General Procedure for Trans-*tert*-butylation of 2,7-Di-*tert*-butyl-4-substituted-pyrenes in the Presence of Nafion-H with Toluene as an Acceptor. A mixture of 2,7-di-*tert*-butyl-4-substituted-pyrenes (200 mg) and Nafion-H (200 mg) in toluene (5 mL) was refluxed until completion of the reaction. Progress was monitored by GLC (2-m OV-1 column). The Nafion-H was then filtered from the cooled mixture, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane) to afford a colorless solid, which was recrystallized from ethanol in all cases.

3a (90%): colorless prisms; mp 150–151 °C (lit.[13] mp 149–150 °C).

3g (86%): colorless prisms; mp 148–151 °C (lit.[14] mp 147.5–148 °C).

3h (81%): colorless prisms; mp 58–62 °C; IR 3050, 2950, 2930, 1600, 1590, 1450, 1370, 1180, 880, 830, 710 cm^{-1}; ^1H NMR δ 1.55 (3 H, t, J = 6.3 Hz), 3.35 (2 H, q, J = 6.3 Hz), 7.9–8.4 (9 H, m); EIMS m/e 230 (M^+). Anal. Calcd for $C_{18}H_{14}$ (M^+): C, 93.87; H, 6.13. Found: C, 93.65; H, 6.26.

3i (72%): colorless oil; IR 3050, 2950, 2930, 1600, 1590, 1450, 1370, 1180, and 880 cm^{-1}; ^1H NMR δ 1.06 (3 H, t, J = 7.3 Hz), 1.96 (2 H, sex, J = 7.3 Hz), 3.20 (2 H, t, J = 7.3 Hz), 7.9–8.3 (9 H, m); high resolution EIMS calcd for $C_{19}H_{16}$ (M^+) 244.1251, found 244.1252.

(12) Lund, H.; Berg, A. *Mathfyth. Medd.* **1946**, 22, 1.

(13) Clar, E. *Chem. Ber.* **1936**, *69*, 1683.

(14) Berg, A. *Acta Chem. Scand.* **1956**, *10*, 1326.

Antineoplastic Agents. 200. Absolute Configuration of the Bryostatins[1]

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By 1755 the phylum Bryozoa was firmly placed in the animal kingdom and three years later the marine bryozoan *Bugula neritina* (Gymnolamota class, contains some 3000 of the 4000 known Bryozoa) was recorded.[2] In 1968, we detected[3] the presence of highly active antineoplastic constituents in this otherwise unpretentious appearing organism. Fourteen years later the isolation and structure of bryostatin 1 (**1a**),[4] the first member of a new class of remarkable antineoplastic,[5] immunopotentiating,[6,7] and

(1) Paper 199 appears as Drexler, H. G.; Gignac, S. M.; Pettit, G. R.; Hoffbrand, A. V. *Eur. J. Immunol.* **1990**, *20*, 119–127.

(2) For leading references, consult: Christophersen, C. *Acta Chem. Scand.* **1985**, B39, 517–529.

(3) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature* **1970** *227*, 962–963.

(4) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. J. *J. Am. Chem. Soc.* **1982**, *104*, 6846–6848.

(5) (a) Pettit, G. R.; Leet, J. E.; Herald, C. L.; Kamano, Y.; Boettner, F. E.; Baczynskyj, L.; Nieman, R. A. *J. Org. Chem.* **1987**, *52*, 2854–2860. (b) Kraft, A. S.; William, F.; Pettit, G. R.; Lilly, M. B. *Cancer Res.* **1989**, *49*, 1287–1293. (c) Drexler, H. G.; Gignac, S. M.; Jones, R. A.; Scott, C. S.; Pettit, G. R.; Hoffbrand, A. V. *Blood* **1989**, *74*, 1747–1757.

(6) (a) Trenn, G.; Pettit, G. R.; Takayama, H.; Hu-li, J.; Sitkovsky, M. V. *J. Immunol.* **1988**, *140*, 433–439. (b) Mohr, H.; Pettit, G. R.; Plessing-Menze, A. *Immunobiol.* **1987**, *175*, 420–430.

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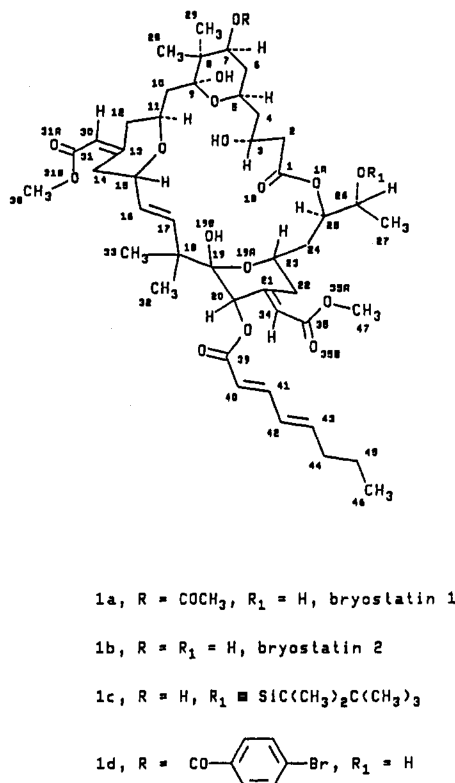


*anti*tumor-promoting[8] substances was summarized. The present investigation was undertaken to increase the availability of bryostatin 1 for clinical development, to fulfill the needs demanded by its increasing use as a biochemical probe,[9] and to establish unequivocally the absolute configuration required for total synthesis. In addition, due to recent advances in high field NMR spectroscopy, it became necessary to reexamine one aspect of the structure assigned eight years ago to bryostatin 3. We report here solutions to these important objectives.

Because the isolated yield of bryostatin 2 (**1b**) from *B. neritina* approximates that of bryostatin 1 (**1a**), it became necessary to find an efficient route for transforming **1b** → **1a**. A very selective high yield (82% overall) chemical conversion was achieved by orchestrating differing steric and reactivity requirements uncovered for the C-7 and C-26 hydroxyl groups of bryostatin 2 (**1b**) as follows. Selective (the C-3 hydrogen atom is relatively unreactive due to hydrogen bonding[4]) protection of the C-26 hydroxyl group of bryostatin 2 (**1b**) with *tert*-butyldimethylsilyl chloride[10] in dimethylformamide containing 4-(*N*,*N*-dimethylamino)pyridine and triethylamine afforded silyl ether **1c**.[11,12] Successive treatment with acetic anhydride–pyridine (room temperature) and 48% hydrofluoric acid–acetonitrile (1:20 at 0–5 °C) provided bryostatin 1 (**1a**), thereby nearly doubling the potential availability of this valuable substance from *B. neritina*. Analogous selective synthesis of bryostatin 2 7-(*p*-bromobenzoate) (**1d**), mp 192–193 °C dec, from bryostatin 2 26-(*tert*-butyldimethylsilyl ether) (**1c**) and *p*-bromobenzoic acid, with subsequent deprotection of the C-26 protecting group, yielded crystals (with difficulty from chloroform–methanol) of a suitable heavy atom containing derivative required for the absolute structure determination employing X-ray crystallographic techniques.

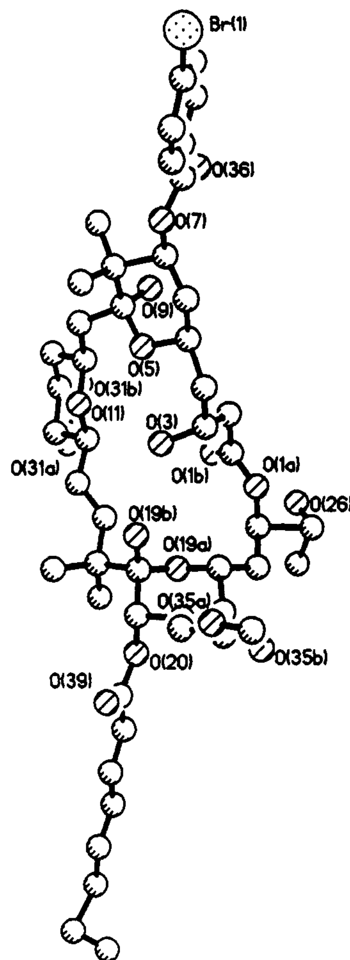


Figure 1. Computer-generated perspective view of bryostatin 2 7-(*p*-bromobenzoate) (**1d**).

Because of the complexity and size of the structure, low temperature (−40 and −75 °C) data collections were initially attempted. But less useful diffraction patterns were obtained, apparently due to crystal cracking or crystal phase transition(s) than for data collected at room temperature (26 ± 1 °C). Assuming our objectives might still be met with data collected at the higher temperatures, X-ray reflections for benzoate **1d** were collected at the latter temperature over one octant. Immediately following each measurement, its Friedel equivalent was measured, when accessible, under identical conditions.

A computer-generated perspective drawing depicting the absolute configuration of bryostatin 2 7-(*p*-bromobenzoate) is shown in Figure 1. The conformation of the macrocyclic ring present in the bryostatin 2 bromobenzoate crystal structure was found to mimic almost precisely the con-

(7) (a) May, W. S.; Sharkis, S. J.; Esa, A. H.; Gebbia, V.; Kraft, A. S.; Pettit, G. R.; Sensenbrenner, L. L. *Cell Biology* **1987**, *84*, 8483–8487. (b) Hess, A. D.; Silanskis, M. K.; Esa, A. H.; Pettit, G. R.; May, W. S. *J. Immunol.* **1988**, *141*, 3263–3269.

(8) (a) Hennings, H.; Blumberg, P. M.; Pettit, G. R.; Herald, C. L.; Shores, R.; Yuspa, S. H. *Carcinogenesis* **1987**, *8*, 1342–1346. (b) Yuspa, S. H.; Hennings, H.; Sako, T.; Pettit, G. R.; Hartley, J.; Blumberg, P. M. In Tumor Promotion: A Problem of Differential Responses of Normal and Neoplastic Cells to Trophic Stimuli, *Anticarcinogenesis and Radiation Protection*; Cerutti, P. A., Nygaard, O. F., Simic, M. G., Eds.; Plenum: New York, 1987.

(9) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci.* **1988**, *85*, 7197–7201.

(10) Ziegler, F. E.; Nangia, A.; Schulte, G. *Tetrahedron Lett.* **1988**, 1669–1672.

(11) Results of high field ^{1}H and ^{13}C NMR combined with high resolution mass spectral interpretations were employed to confirm the structure of each synthetic intermediate.

(12) For details of the silyl ether (**1c**) synthesis, refer to Pettit, G. R.; Sengupta, D.; Herald, C. L. *Can. J. Chem.* Submitted for publication.

formation displayed by bryostatin 1 (**1a**).[4] Slight differences in the conformations assumed by the octa-2,4-dienoic acid side chains of the two bryostatins were noted. With bryostatin 2 *p*-bromobenzoate (**1d**), the long chain assumed a more linear conformation, lacking the "kinks" displayed in the previously reported bryostatin 1 crystal structure.[4] In addition, the bromobenzoate ring adopted a perpendicular orientation with respect to the overall macrocyclic ring and to its pyran ring attachment. By contrast, the C-7 acetyl group in bryostatin 1 (**1a**) assumes a more planar orientation with respect to these two rings.

Abnormal bond distances and/or large thermal parameters were encountered in three regions of the molecule. One region, consisting of the side chain at C25, had bond distances (C25–C26 1.77 Å and O26–C26 1.24 Å) deviating from the expected norm. Very large thermal parameters were noted for O31A and C38, as well as the terminal three atoms of the octadienoic acid side chain (C44, C45, and C46). Such large thermal parameters suggested possible disorder in the latter two regions. Subsequent analysis of Fourier difference maps revealed small, residual electron densities at alternate sites near the terminal atoms of the dienoic acid side chain, suggesting other conformations were possible. However, alternate (and chemically reasonable) conformational coordinates could not be reliably assigned. No significant residual electron densities were noted for the regions near atoms O31A and C38. Thermal effects causing conformational variations in these regions presumably account for the abnormalities and instabilities noted during refinement. These problems disappeared in the first two regions at the lower temperatures used in the case of bryostatin 1.[4,13]

Absolute configurational assignments for bryostatin 2 (**1b**) could be readily ascertained from its bromobenzoate derivative (**1d**), based primarily upon the pronounced anomalous scattering effects produced by bromine in the latter. Standard crystallographic residuals (*R* factors) obtained for the model depicted for **1d** were $R = 0.114$ and $R_w = 0.097$ over the observed data and *R* values obtained for the enantiomeric image of bromobenzoate **1d** were $R = 0.118$ and $R_w = 0.101$. These residuals are slightly high for diffractometer data[14] and partially reflect the mediocre quality of the crystal as well as a suspected less-than-adequate empirical absorption correction.

The differences between the *R* values for the two enantiomers are significant. On the basis of Hamilton's *R*-factor significance test,[15] the probability is >99.9% that the absolute configuration for bryostatin **1d** is that represented by the structure shown. For additional confirmation, the absolute structure index η described by Rogers[16] was also determined. The Rogers index has the particular advantage of being nearly independent of experimental conditions, including inadequate or uncorrected absorption.[17,18] Refinement[18] of η to +1 with small estimated standard deviation (esd) is indicative of a correct choice of the absolute configuration for the model under consideration. On the other hand, refinement to a −1 indicates that the structure should be inverted to the enantiomeric image of the model. In the case of the bryostatin **1d**, refinement[19] of η resulted in a value of 1.079 (esd 0.097), indicating that the absolute configuration shown (Figure 1) is correct. Thus, the absolute stereochemical assignments of the chiral centers tentatively selected for bryostatin 1[4] (based upon the much smaller anomalous dispersion effects of oxygen and carbon) are in complete agreement with the current assignments determined for bryostatin 2. Consequently, the stereochemical designations for the 11 chiral centers in both bryostatins 1 (**1a**) and 2 (**1b**) can now be unambiguously defined as follows: 3*R*, 5*R*, 7*S*, 9*S*, 11*S*, 15*R*, 19*S*, 20*S*, 23*S*, 25*R*, 26*R*.

A reinvestigation of the structure earlier assigned as bryostatin 3[20] by employing high field NMR techniques led to a reassignment in the C-21–C-23 and C-34–C-35 regions to yield revised structure **2**. Since bryostatin 3 is biogenetically related to bryostatin 1, the chiral centers

Table I. ^{13}C (100 MHz), ^{1}H (400 MHz), and HMBC (500 MHz) NMR Data for Bryostatin 3 Recorded in CD_3CN (J_{H-H} in Hertz in Parentheses)

				HMBC
C-1	172.28	H-2	2.53 t (11)	C-1
2	42.32	2′	2.49 br d (11)	C-1
3	69.69	3	4.05 br m	
4	40.56	4	1.77 br t (13)	
5	66.63	4′	1.67 br d (13)	
6	34.05	5	4.20 br dd (11, 13)	
7	73.23	6	1.64 br dd (5, 12)	C-5, C-7
8	42.04	6′	1.44 ddd (11, 12, 12)	
9	102.62	7	5.12 dd (5, 12)	
10	42.72	10	1.99 dd (7, 15.8)	C-9, C-11
11	73.23	10′	1.70 br d (15.8)	C-9, C-12
12	44.46	11	3.92 br dd (7, 14)	
13	158.46	12	2.15[a]	C-13
14	37.01	12′	2.15[a]	C-13
15	79.64	14	3.57 br d (14)	C-13
16	133.00	14′	1.87 br dd (12, 14)	C-13
17	138.15	15	4.16 br dd (8.5, 12)	
18	46.05	16	5.39 dd (8.4, 16)	C-18
19	102.49	17	5.76 d (16)	C-15, C-18
20	69.32	20	5.87 br s	C-21, C-1″, C-19
21	169.09	22	4.61 br d (9)	C-20, C-21, C-24
22	82.18	23	3.72 br dd (9, 11)	
23	70.08	24	2.25 br dd (11, 14)	
24	32.90	24′	1.90[a]	
25	72.67	25	5.08 br dd (5, 11)	
26	68.47	26	3.84 br m	
27	18.63	27	1.08 d (6.4)	C-25, C-26
28	21.14	28	0.88 s	C-7, C-8, C-9, C-29
29	17.20	29	0.98 s	C-7, C-8, C-9, C-28
30	114.60	30	5.75 br s	C-31
31	167.61	32	0.98 s	C-17, C-18, C-19, C-33
32	21.24	33	1.13 s	C-17, C-18, C-19, C-32
33	24.73	34	5.81 br s	C-22, C-35
34	114.33	OMe	3.65 s	C-31
35	172.64	OAc	1.98 s	C-1′
36	51.56	OR″		
OAc	171.39	2″	5.92 d (15.3)	
2′	21.42	3″	7.35 br dd (9.5, 15)	
OR″	166.67	4″	6.30[a]	C-5″, C-6″
2″	118.93	5″	6.30[a]	C-6″
3″	147.86	6″	2.15[a]	C-4″, C-5″
4″	129.21	7″	1.45 m	C-5″, C-6″, C-8″
5″	147.42	8″	0.91 t (7.3)	C-6″, C-7″
6″	35.65			
7″	22.57			
8″	13.93			

[a] These signals were overlapped and couplings not measured.

(13) For more detail, see: Arnold, E. Ph.D. Dissertation, Cornell University, Ithica, NY, 1982. However, some disorder was still observed for the terminal carbon atoms of the dienoic acid side chain of bryostatin 1, even at the lower (−100 °C) temperature.

(14) Refinement of "well-behaved" structures should be capable of refinement to an *R* value of below 0.1, and most below 0.06. Stout, G. H.; Jensen, L. H. *X-Ray Structure Determination-A Practical Guide*, John Wiley & Sons, Inc.: New York, 1989; p 230.

(15) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502–510.

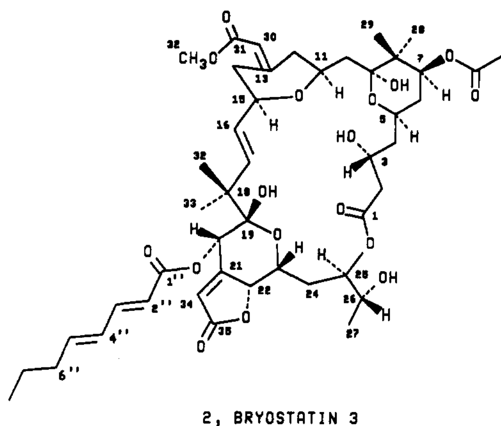
(16) Rogers, D., *Acta Crystallogr.* 1981, *A37*, 734–741.

(17) Bernardinelli, G.; Flack, H. D. *Acta Crystallogr.* **1987**, *A43*, 75–78.

(18) Jones, P. G.; Meyer-Bäse, K. *Acta Crystallogr.* **1987**, *A43*, 79–80.

(19) Refinement of the Rogers' η parameter and preparation of supplementary tables were performed with SHEXTL-PLUS, G. Sheldrick, Siemens Analytical X-Ray Instruments, Inc., Madison, WI 53719.

(20) Pettit, G. R.; Herald, C. L.; Kamano, Y. *J. Org. Chem.* **1983**, *48*, 5354–5356.



2, BRYOSTATIN 3

at C-20 and C-23 are presumably the same. The stereochemistry at C-22 of bryostatin 3 was assigned with NOE difference spectroscopy results: irradiation on the resonance of H-20 (δ 5.87) enhanced H-33 (δ 1.13) and H-22 (δ 4.61); irradiation on H-22 enhanced H-23 and H-20; irradiation on H-23 enhanced the H-22 and a signal at δ 5.68 (C-19 OH). These NOE results indicate that H-20, H-22, and H-23 are all above the pyran ring; therefore, H-22 is assigned the β configuration. The otherwise minor revision to a five-membered lactone versus the earlier six-membered lactone involving the C-19 hydroxy group is very important for various biochemical mechanistic reasons.[9] The heteronuclear molecular bond correlations (HMBC) summarized in Table I were crucial to assigning structure 2 to bryostatin 3.

The advances in bryostatin chemistry summarized here will simplify a number of on-going chemical and biological investigations in this important field.

Experimental Section[12]

General Procedures. All chromatographic solvents were redistilled. Commercial sources of silica gel (E. Merck, Darmstadt, 70–230 mesh) uniplates (Analtech, Inc., Newark, DE) were used for thin layer chromatography (TLC). The TLC plates were viewed with UV light and developed with anisaldehyde–sulfuric acid spray reagent followed by heating. The NMR spectra were measured on a Bruker AM-400 instrument, with an ASPECT 3000 computer, with $CDCl_3$ employed as solvent, and on a Varian VXR-500S instrument with a Sun 4/260 computer.

Bryostatin 2 7-(*p*-Bromobenzoate) (1d). To a solution of bryostatin 2 26-(*tert* butyl dimethyl silyl ether) (4.6 mg)[12] in CH_2Cl_2 (150 μL) were added *p*-bromobenzoic acid (1.5 mg), dicyclohexylcarbodiimide (3.0 mg), and 4-pyrrolidinopyridine (0.8 mg). The mixture was stirred at room temperature for 2 h. The *N,N*-dicyclohexylurea was collected and the filtrate dried. The residue was purified by silica gel column chromatography (1:1 hexane–ethyl acetate) to afford bryostatin 2 7-(*p*-bromobenzoate) 26-(*tert* butyl dimethyl silyl ether) (5.0 mg, 92%). The silyl ether group was removed by treatment with 1:20 48% hydrochloric acid–acetonitrile (250 μL, 0–5 °C, 4 h), solvent evaporated, and product purified by silica gel (1:1 hexane–ethyl acetate) column chromatography. Bryostatin 2 7-(*p*-bromobenzoate) (2.7 mg, 60%) was recrystallized from CH_2Cl_2–CH_3OH: mp 192–193 °C dec; α^{25}_D = +10° (2 mg/mL, CH_3OH); UV $\lambda^{CH_3OH}_{max}$ 243 mμ (ϵ 6430); IR (thin film) 3440, 2900, 1715, 1625, 1565, 1437, 1310, 1250, 1152, 1085 cm^{-1}. The high resolution (400 MHz) proton NMR spectrum was as expected for bryostatin 2 7-(*p*-bromobenzoate).

Crystal Structure Determination of Bryostatin 2 7-(*p*-Bromobenzoate) (1d): molecular formula $C_{52}H_{69}O_{17}Br$, F.W. 1046.01, *F*(000) 2208, space group $P2_12_12_1$, crystal dimensions 0.26 × 0.24 × 0.40 mm, radiation, Cu Kα, λ = 1.541 84 Å, temperature 26 ± 1 °C, cell constants *a*, 12.999 (2) Å, *b*, 19.947 (4) Å, and *c*, 21.641 (4) Å, *V* = 5611.8 Å^3, *Z* = 4, ρ_o = 1.237 g/cm^3, ρ_c = 1.238 g/cm^3, and μ = 15.24 cm^{-1}. **Collection parameters:** instrument Enraf-Nonius, CAD4 diffractometer; monochromator graphite crystal incident beam; attenuator Ni foil, factor = 11.7; take-off angle 2.0°; detector aperture 1.8 mm horizontal, 4.0 mm vertical; crystal detector distance 21 cm; scan type ω–2θ, scan rate 0.8 to 5.5°/min (in ω); scan width 0.8 + 0.15 tan θ deg; maximum 2θ 150.0°; and number of reflections measured, one octant + Friedels, 12 159 total, 9715 unique. **Corrections made:** Lorentz-polarization, ϕ scan empirical absorption (0.979 to 0.999 on F_o), linear decay (0.987 to 1.000 on F_o), and anisotropic decay (0.871 to 1.351 on F_o). **Solution and refinement:** direct methods structure solution was accomplished by means of SHELXS-86: Sheldrick, G. Institut für Anorganische Chemie der Universität, Tammannstrasse 4, D-3400 Göttingen, Federal Republic of Germany, using the TEXP feature for partial structure expansion until all 70 non-hydrogen atoms in the molecule were located. All least-squares block-diagonal refinement calculations were performed by using the CRYSTALS computing package: Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W., 1985, Chemical Crystallography Laboratory, University of Oxford, Oxford, OX1 3PD, England. Due to instability noted during initial refinements, the Robust–Resistant (Tukey and Prince) weighting scheme option was used until convergence occurred, then the weighting scheme was changed to $1/\sigma^2(F_o)$ for the final cycles of refinement. Final steps of refinement were done with 5890 reflections in which $F_o^2 > 2\sigma(F_o^2)$. All non-hydrogen atoms (with the exception of the atoms C38 and C43–46) were refined anisotropically. Hydrogen atom coordinates were calculated with fixed thermal parameters (U_{iso} = 0.08 Å^2). They were included but not refined.

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Supplementary Material Available: Table of atomic coordinates and isotropic displacement parameters and 400-MHz ^{1}H NMR spectrum for bryostatin 2 7-(*p*-bromobenzoate) (4 pages). Ordering information is given on any current masthead page.

Synthesis of Chiral α-Alkyl Phenethylamines via Organometallic Addition to Chiral 2-Aryl-1,3-oxazolidines

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Introduction

In order to fulfill a supply requirement for an optically active pure pharmaceutical candidate, we required substantial quantities of (*R*)-α-methyl-*p*-bromophenethylamine on a continual basis. Normally, this class of amines is readily available for small-scale use, but the commercial availability of large quantities is severely limited. We therefore sought an alternative synthesis that would permit facile access to these amines in high optical purity in a manner that would be amenable to pilot-plant scale. Many of the published routes to α-alkyl phenethylamines require a tedious resolution of the corresponding racemate.[2] The

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