C-25 and C-41), 174.8 (s, C-42), 50.6 (q, 42-OMe), 175.3 (s, C-43), 51.6 (q, 43-OMe), 171.3 (s, C-44), and 52.0 (q, 44-OMe); EIMS m/z 761 (M^+), 729, 701, and 671.

8,9-Dihydroxy-43-*O*-methylmanzamenone A (1c). To the solution of 43-*O*-methylmanzamenone A (**1a**, 26.6 mg, 0.035 mmol) in THF (1.2 mL) and pyridine (0.3 mL) was added 10.5 mg (0.041 mmol) osmium tetraoxide in THF (105 μL), and the mixture was stirred for 2 h at room temperature. After addition of saturated aqueous sodium bisulfite solution (1.6 mL), stirring was continued for 1 h. Then the mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by a silica gel column with acetone/hexane (1:3) to give the 8,9-diol **1c** (27.1 mg): ^{1}H NMR ($CDCl_3$) δ_H 4.08 (1 H, dd, J = 7.3 and 5.8 Hz; H-1), 3.54 (1 H, d, J = 5.8 Hz; H-2), 4.98 (1 H, d, J = 4.0 Hz; H-4), 3.88 (1 H, dd, J = 11 and 4.0 Hz; H-5), 3.61 (1 H, dd, J = 11 and 7.3 Hz; H-6), 1.87 (1 H, m; H-10a), 2.09 (1 H, m; H-10b), 2.41 (1 H, ddd, J = 13, 9.5, and 5.2 Hz; H-26a), 3.02 (1 H, ddd, J = 13, 9.5, and 6.3 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.7 Hz; H_3-25 and H_3-41), 3.79, 3.74, and 3.53 (each 3 H, s; 42-OMe, 43-OMe, and 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 42.8 (d, C-1), 45.9 (d, C-2), 132.3 (s, C-3), 125.3 (d, C-4), 41.6 (d, C-5), 44.1 (d, C-6), 93.2 (s, C-8), 91.9 (s, C-9), 39.4 (t, C-10), 31.9, 30.4, 30.3, 29.7 (many carbons overlapped), 27.7, 23.0, and 22.7 (each t, C-11–C-24 and C-26–C-40), 14.1 (2 C, q, C-25 and C-41), 173.2, 172.3, and 164.1 (each s, C-42, C-43, and C-44), 51.9, 51.6, and 51.5 (each q, 42-OMe, 43-OMe, and 44-OMe); FABMS m/z 896 (M + diethanolamine + H)$^+$, 864, and 846.

3,4-Dihydroxy-43-*O*-methylmanzamenone B (2b). 43-*O*-Methylmanzamenone B (**2a**, 6.2 mg, 0.008 mmol) was treated with OsO_4 (2.5 mg, 0.010 mmol) by the same procedure as above to afford the 3,4-diol **2b** (1.9 mg): ^{1}H NMR ($CDCl_3$) δ_H 3.66 (1 H, dd, J = 11 and 6.7 Hz; H-1), 2.11 (1 H, d, J = 11 Hz; H-2), 2.48 (1 H, s; 3-OH), 3.70 (1 H, dd, J = 6.7 and 3.0 Hz; H-4), 3.82 (1 H, d, J = 3.0 Hz; 4-OH), 3.25 (1 H, dd, J = 11 and 6.7 Hz; H-5), 3.36 (1 H, t, J = 6.7 Hz; H-6), 1.12 (1 H, m; H-10a), 2.02 (1 H, m; H-10b), 1.93 (1 H, ddd, J = 13, 9.0, and 5.0 Hz; H-26a), 3.22 (1 H, ddd, J = 13, 10, and 5.5 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.7 Hz; H_3-25 and H_3-41), 3.77, 3.79, and 3.82 (each 3 H, s; 42-OMe, 43-OMe, and 44-OMe); FABMS m/z 896 (M + diethanolamine + H)$^+$ and 864.

Ester Exchange of Manzamenone C (3). Manzamenone C (**3**, 0.1 mg) was treated with 2 N HCl/MeOH (0.1 mL) at room temperature for 10 min. After evaporation of the solvent, the residue was analyzed by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: MeOH/$CHCl_3$ (7:3); flow rate: 2.0 mL/min; detection: UV (254 nm)] to show a peak at t_R 20 min together with a small peak at t_R 22 min in the ratio of 8:1, which were ascribed to 43-*O*-methylmanzamenone A (**1a**) and the starting material **3**, respectively.

Determination of Amino Acid Residue of Manzamenone E (5). Manzamenone E (**5**, 50 μg) was heated in 6 N HCl at 110 °C for 24 h. The hydrolyzate was analyzed by a Hitachi amino acid autoanalyzer (Model 835) to show the presence of valine (t_R 55.1 min). For the chiral GC analysis, the acid hydrolysate of manzamenone E (**5**, 50 μm) was treated with 10% HCl/MeOH (0.5 mL) at 100 °C for 30 min. After the reaction mixture was evaporated under vacuum, the residue was heated in a mixture of trifluoroacetic anhydride (0.3 mL) and CH_2Cl_2 (0.3 mL) at 100 °C for 5 min and then evaporated. The residue was dissolved in CH_2Cl_2 and subjected to capillary GC analysis [Chirasil-Val column (Alltech, 0.32 mm × 25 m); carrier gas: nitrogen; program rate: 50–200 °C at 4 °C/min] to show a peak at t_R 6.6 min, which was ascribed to L-valine by comparison with the peaks of TFA/Me derivatives of authentic D- and L-valines (t_R 6.2 and 6.6 min, respectively).

Ester Exchange of Manzamenone F (6). Manzamenone F (**6**, 1 mg) was treated with 2 N HCl/MeOH (5 mL) under reflux for 10 min. After evaporation of the solvent, the residue was analyzed by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: CH_3CN/$CHCl_3$ (7:3); flow rate: 2.0 mL/min; detection: UV (254 nm)] to show a peak at t_R 32.6 min together with a small peak at t_R 29.0 min in the ratio of ca. 9:1, which were ascribed to 43-*O*-methylmanzamenone A (**1a**) and 43-*O*-methylmanzamenone B (**2a**), respectively. The major product **1a** was isolated by HPLC (the same conditions as above) and firmly identified by comparison of TLC [R_f 0.45, hexane/EtOAc (3:1)], ^{1}H NMR, and EIMS data with those of authentic sample. After reflux for 2 h under the same conditions, the ratio of the products (**1a** and **2a**) was shown to be ca. 1:1 by HPLC analysis.

3-Carboxy-5-(carboxymethyl)-4-tetradecyl-1-oxacyclopent-3-en-2-one (7): a colorless oil; $[\alpha]^{25}_D$ +12° (c 0.78, $CHCl_3$); IR ($CHCl_3$) 3200, 1740, and 1720 cm^{-1}; UV (MeOH) λ_{max} 235 nm (ϵ 5500); ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.53 (1 H, dd, J = 16 and 8.9 Hz; H-6a), 2.89 (1 H, dd, J = 16 and 3.6 Hz; H-6b), 2.13 (1 H, ddd, J = 15, 9.0, and 6.0 Hz; H-9a), 2.49 (1 H, ddd, J = 15, 9.0, and 7.1 Hz; H-9b), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.9 Hz; H_3-22); ^{13}C NMR ($CDCl_3$) δ_C 173.4 (s, C-2), 137.7 (s, C-3), 133.8 (s, C-4), 76.8 (d, C-5), 24.5 (t, C-6), 173.4 (s, C-7), 170.0 (s, C-8), 29.7 (many carbons overlapped), 27.1, and 22.7 (each t, C-9–C-21), and 14.1 (q, C-22); EIMS m/z 382 (M^+), 338, and 293; HREIMS m/z 382.2372, calcd for $C_{21}H_{34}O_6$ (M) 382.2355.

Dimethyl Ester 7a. Compound **7** (0.5 mg) in methanol (0.5 mL) was treated with diazomethane in ether (1 mL) at room temperature for 20 min. After evaporation of the solvent, the residue was purified by a silica gel column chromatography (0.5 × 4 cm) with $CHCl_3$ to afford the dimethyl ester **7a** (0.5 mg): ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.53 (1 H, dd, J = 16 and 8.9 Hz; H-6a), 2.89 (1 H, dd, J = 16 and 3.6 Hz; H-6b), 2.13 (1 H, ddd, J = 15, 9.0, and 6.0 Hz; H-9a), 2.49 (1 H, ddd, J = 15, 9.0, and 7.1 Hz; H-9b), 3.74 and 3.95 (each 3 H, s; 7-OMe and 8-OMe), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.6 Hz; H_3-22); EIMS m/z 410 (M^+).

Di-*p*-bromophenacyl Ester (7b). Compound **7** (2.0 mg) was treated with *p*-bromophenacyl bromide (8.0 mg) in dimethylformamide (0.2 mL) containing potassium fluoride (3.0 mg) at room temperature for 2 h. After addition of H_2O (0.5 mL), the reaction mixture was extracted with ether (1 mL × 3). The ether layer was washed with H_2O (0.5 mL × 5), dried over sodium sulfate, and evaporated under reduced pressure. The residue was purified by a silica gel column chromatography (1.1 × 4 cm) with hexane/ether (1:1) to give the *p*-bromophenacyl ester **7b** (0.6 mg): UV (EtOH) λ_{max} 255 nm (ϵ 37 000); CD (EtOH) λ_{ext} 247 nm ($\Delta\epsilon$ +25) and 227 (−17); ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.72 (1 H, dd, J = 16 and 8.2 Hz; H-6a), 3.06 (1 H, dd, J = 16 and 4.0 Hz; H-6b), 2.23 (1 H, m; H-9a), 2.69 (1 H, m; H-9b), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.6 Hz; H_3-22), 5.27, 5.41, 5.54, and 5.64 (each 1 H, d, J = 17 Hz), 7.61 and 7.64 (each 2 H, d, J = 8.3 Hz), and 7.75 (4 H, d, J = 8.3 Hz); EIMS m/z 776 (M^+).

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Supplementary Material Available: All spectra of **1a** and **7** and ^{1}H NMR spectra of manzamenones A–F (**1–6**) (24 pages). This material is contained in many 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.

Bryostatins Revisited: A New Bryostatin 3 and the Use of NMR To Determine Stereochemistry in the C-20–C-23 Area

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During our continued isolation studies of the bryostatins present in lyophilized *B. neritina*, a new compound, **1**, was isolated. It has the same exact mass (by HR FABMS) as

Table I. ^{1}H NMR Chemical Shifts[a] of Bryostatin 3 (1), 20-*epi*-Bryostatin 3 (2),[b] Bryostatin 1 (3), and Bryostatin 2 (4)

proton no.	1			2			3			4		
	δ	mult	J (Hz)	δ	mult	J (Hz)	δ	mult	J (Hz)	δ	mult	J (Hz)
190H	5.41	s		5.77	d	1.8	5.15	br s		5.19	bs	
20	5.76	s		5.87	dt	0.6, 1.8	5.18	s		5.20	s	
34	6.08	d	1.8	5.85	t	1.8	6.00 [5.89][c]	d [d]	1.8 [2.0]	6.01	d	2.0
22ax	4.56	dd	9.0, 1.8	4.47	ddd	9.0, 1.7, 0.6	2.06 [2.09]	m [ddd]	[13.8, 11.3, 2.0]	2.07	ddd	13.8, 11.3, 2.7
22eq							3.69 [3.63]	m [dd]	[13.8, 2.5]	3.69	dd	13.8, 2.3
23	3.75	ddd	11.5, 9.0, 2.5	3.77	ddd	11.4, 9.0, 2.4	4.02 [4.01]	tt [tt]	11.3, 2.4 [11.3, 2.4]	4.02	tt	11.3, 2.5
24a	2.40	ddd	14.2, 12.1, 2.2	2.39	ddd	14.3, 12.3, 2.3	1.83	ddd	13.8, 11.6, 2.9	1.83	ddd	13.9, 11.2, 2.9
24b	1.95	ddd	14.1, 11.4, 2.9	1.88	ddd	14.2, 11.4, 3.1	1.99 [1.99]	m [ddd]	[14.0, 12.5, 2.8]	1.96	ddd	13.9, 12.1, 2.6
25	5.14	ddd	12.2, 5.5, 2.9	5.06	ddd	12.4, 5.5, 3.0	5.17 [5.23]	m [ddd]	[12.2, 4.1, 2.6]	5.19	ddd	12.2, 5.6, 3.0
26	3.76	dq	5.6, 6.4	3.82	dq	5.8, 6.5	3.78 [3.85]	m [dq]	[4.3, 6.5]	3.80	dq	5.6, 6.4

[a] $CDCl_3$, 500 MHz. H-16, H-33, H-40, and H-41 show proton shift differences less than 0.1 ppm. [b] Additional coupling information has been extracted by resolution enhancement or spin simulation. [c] [], recorded in CD_3OD.

Table II. ^{13}C NMR Chemical Shifts[a] of Bryostatin 3 (1), 20-*epi*-Bryostatin 3 (2), Bryostatin 1 (3), and Bryostatin 2 (4)

carbon no.	δ			
	1	2	3	4
19	99.59	101.65	99.02	99.02
20	69.94	68.57	74.09	74.05
34	117.11	114.31	119.57	119.57
22	79.47	81.23	31.32	31.28
23	70.32	69.08	64.71	64.67
24	33.20	33.19	35.93	35.86
25	72.86	72.91	73.68	73.78
26	69.86	69.70	70.15	70.18

[a] $CDCl_3$, 125 MHz. Carbon atoms 16, 17, 32, and 33 have changes in chemical shifts less than 3 ppm.

a previously isolated bryostatin 3 (2). Compound 2 was erroneously reported by our group[1] to have structure 1 and by Pettit's group[2] to have structure 1 with cis geometry between H-22 and H-23. In keeping with the published X-ray structures of bryostatin 1 (3)[3] and bryostatin 2 (4),[2] we have named compound 1 bryostatin 3 and renamed compound 2 20-*epi*-bryostatin 3.

Exhaustive 1D and 2D (TOCSY, DQCOSY, NOESY, ROESY, HMQC, and HMBC (4 and 8 Hz)) NMR studies of 1 and 2 have established the structures as shown in Figure 1 and provided an NMR technique for distinguishing between isomers at C-20 in other bryostatins. Quantitative 2D NOE studies were necessary to verify the stereochemistry at C-20 and to establish the stereochemistry as trans between H-22 and H-23 in both isomers of bryostatin 3.

The one-dimensional ^{1}H and ^{13}C NMR spectra for compounds 1 and 2 differ by no more than 0.05 and 0.5 ppm, respectively, except in the area surrounding C-20. In Tables I and II, 1D NMR comparisons have been given between bryostatin 3 (1), 20-*epi*-bryostatin 3 (2), bryostatin 1 (3),[4] and bryostatin 2 (4) for the area surrounding C-20.

While the proton chemical shift differences in Table I are significant, the coupling data afforded more reliable arguments. In three solvents ($CDCl_3$, C_3DCN, and CD_3OD), J values in the C-20 area were constant, indicating no conformational change; in contrast, chemical shifts changed with solvents and concentration. If the C-20 stereochemistry is bryostatin 1-like, which was established by X-ray,[3] then no spin–spin coupling (J) is observed between H-34 and H-20, H-20 and 19-OH, and H-20 and H-22. However, if C-20 is epimerized to *epi*-bryostatin

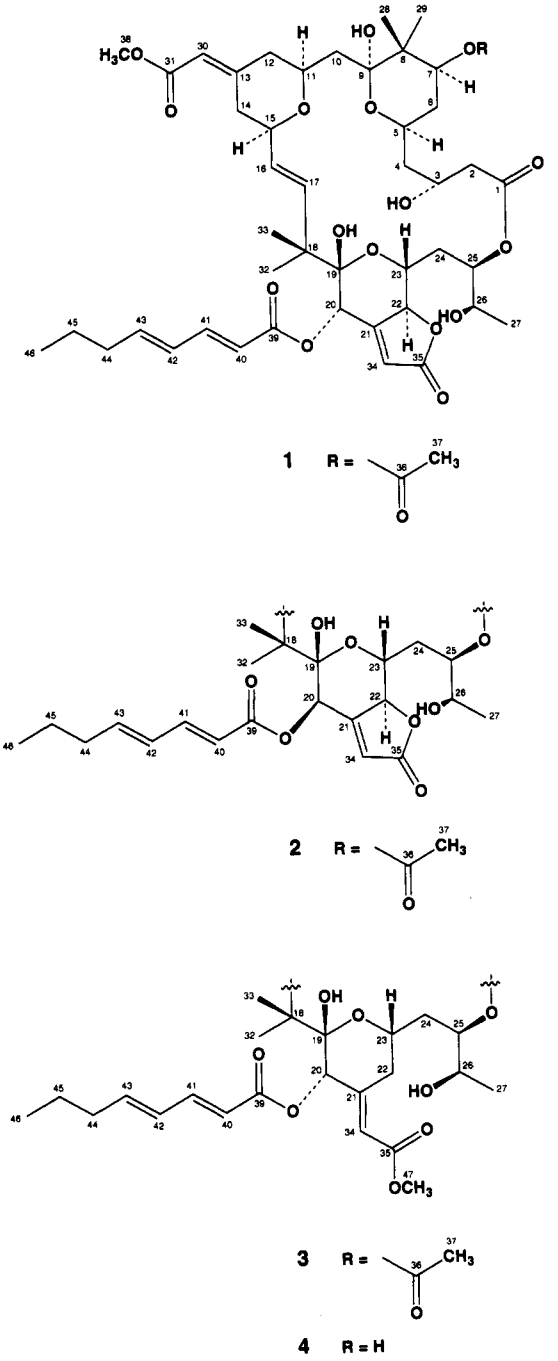

Figure 1. Structures of bryostatin 3 (1), 20-*epi*-bryostatin 3 (2), bryostatin 1 (3), and bryostatin 2 (4).

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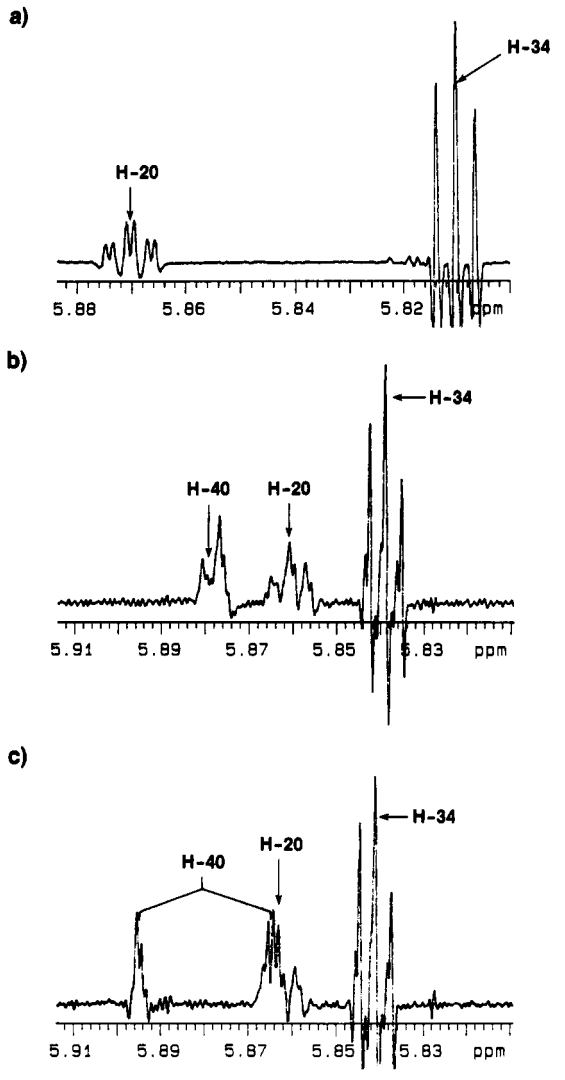

Figure 2. 500-MHz proton 1D NMR spectrum of 20-*epi*-bryostatin 3 (**2**) showing H-20 (a) in CD_3CN, (b) in $CDCl_3$, with irradiation of H-41 at 7.39 ppm to reduce the coupling to H-40 and, therefore, to reveal H-20 without overlap (note the Bloch–Siegert shift in resonances), and (c) in $CDCl_3$, without irradiation at H-41.

3-like, then a J of 1.8 Hz is observed from H-34 to H-20 and H-22, and from H-20 to 19-OH. In this isomer, a smaller "W"[5] coupling of 0.6 Hz is also observed from H-20 to H-22 (Figure 2). In $CDCl_3$, H-20 of the epi isomer **2** was obscured by half of H-40 as shown in Figure 2c. A reduced-coupling experiment (partial decoupling of H-41, Figure 2b) revealed H-20 clearly. Figure 2a, obtained from the same sample as in Figure 2b and 2c by evaporating the $CDCl_3$ and dissolving the sample in CD_3CN showed H-20 clearly with the same "epi"-like couplings. Comparison of this ^1H NMR spectrum in CD_3CN with Pettit's table of NMR shifts established that our 20-*epi*-bryostatin 3 (**2**) is identical to his bryostatin 3.[2] Quantitative NOE data presented below corroborated the C-20 stereochemistries of **1** and **2**.

The stereochemistry between H-22 and H-23 was shown to be trans in both **1** and **2** based on the following evidence. A $^3J_{HH}(22,23)$ of 9 Hz in both isomers in CD_3CN as well as in $CDCl_3$ indicates a trans stereochemistry. The $^3J_{HH}(22,23)$ couplings in **3** and **4**, for which both X-ray structures are known, were 11.3 and 2.5 Hz for trans (axial–axial) and cis (axial–equatorial), respectively. In addition, an average $^3J_{trans}$ of 9.5 Hz and $^3J_{cis}$ of 2.0 Hz in rapamycin, which has a six-membered ring similar to that in bryostatin 1, has been reported.[6] Quantitative NOE proton–proton internuclear distances from both C-20 isomers corroborated the trans geometry at C-22/C-23, supporting the conclusion that the dihedral angle has not changed.

The ROESY buildup experiment was chosen (see Experimental Section) to provide quantitative internuclear proton–proton distances in $CDCl_3$ for **1** and **2** (Table III). The average initial NOE buildup rate from the well resolved methylene pairs on carbon atoms 4, 6, 10, 14, and 24 (Table IIIb) of 920 s^{-1} and the average Dreiding model measured H–H distance of 1.8 Å between these CH_2 pairs were used to quantify NOE buildup rates. For an assumed chair ring, these values tested well on the C-5–C-8 pyran ring (Table IIIc). The reproducibility of the initial buildup rates for proton groups in areas of known geometry in both **1** and **2** gave credence to the data interpretation from ring C-19 to C-23 and H-34. The "anti" H-20/H-22 geometry in **1** resulted in a predicted NOE H–H distance of 4.2 Å (Dreiding model, 3.5 Å), while the "syn" isomer **2** gave 3.3 Å (Dreiding model, 2.67 Å). The relative buildup rates are in agreement with our assignments. The lack of absolute agreement with the Dreiding model and the smaller 9 Hz $J_{axial-axial}$ could indicate that this ring is slightly twisted. Although the H-20/H-34 NOE is obscured by chemical shift overlap, it can be seen from Table III that the C-20 stereochemistry is correct. The H-23/19-OH measured distances provided an interesting C-20 stereochemical check. If hydrogen bonding occurs between 19-OH and the ester function of the octadienyl side chain in 20-*epi*-bryostatin 3 (**2**) then the Dreiding model measured H-23–19-OH distance is 2.85 Å, which compares well with the NOE calculated distance of 2.87 Å. In the absence of this hydrogen bonding in bryostatin 3 (**1**), the 19-OH can freely rotate and approach H-23 to within 1.4 Å; the NOE calculated distance was 2.37 Å, which agrees well with the H-23/19-oxygen distance of 2.5 Å (see Table III).

The ROESY buildup rate for H-22/H-23 in both isomers, while negative, corroborates the trans geometry about this bond in both **1** and **2**. (Negative rate = same phase as the diagonal; positive rate = opposite phase to the diagonal.) These cross peaks indicate that the NOE does not arise from a direct transverse cross correlation, which would be expected from the cis geometry. The H–H distance in the cis isomer measured 2.1 Å (Dreiding model) and would be expected to produce a positive ROESY cross peak. The only discrepancy to Pettit's 1D NOE experiment appears to be for H-22/H-23 (for percentages see Table III). We have no explanation except that our 1D H-22/H-23 enhancement in CD_3CN was too small to be reliably observed; this also indicates a trans geometry about the H-22/H-23 vicinal bond.

We have established from NMR data that the structures for the two bryostatin 3 isomers are **1**, bryostatin 3, and **2**, 20-*epi*-bryostatin 3, and have presented NMR techniques for distinguishing between them. The application of an X-ray determined stereochemistry obtained from a single compound to assign the structures of a presumably homologous series of compounds isolated from the same organisms is commonly used in natural product structure

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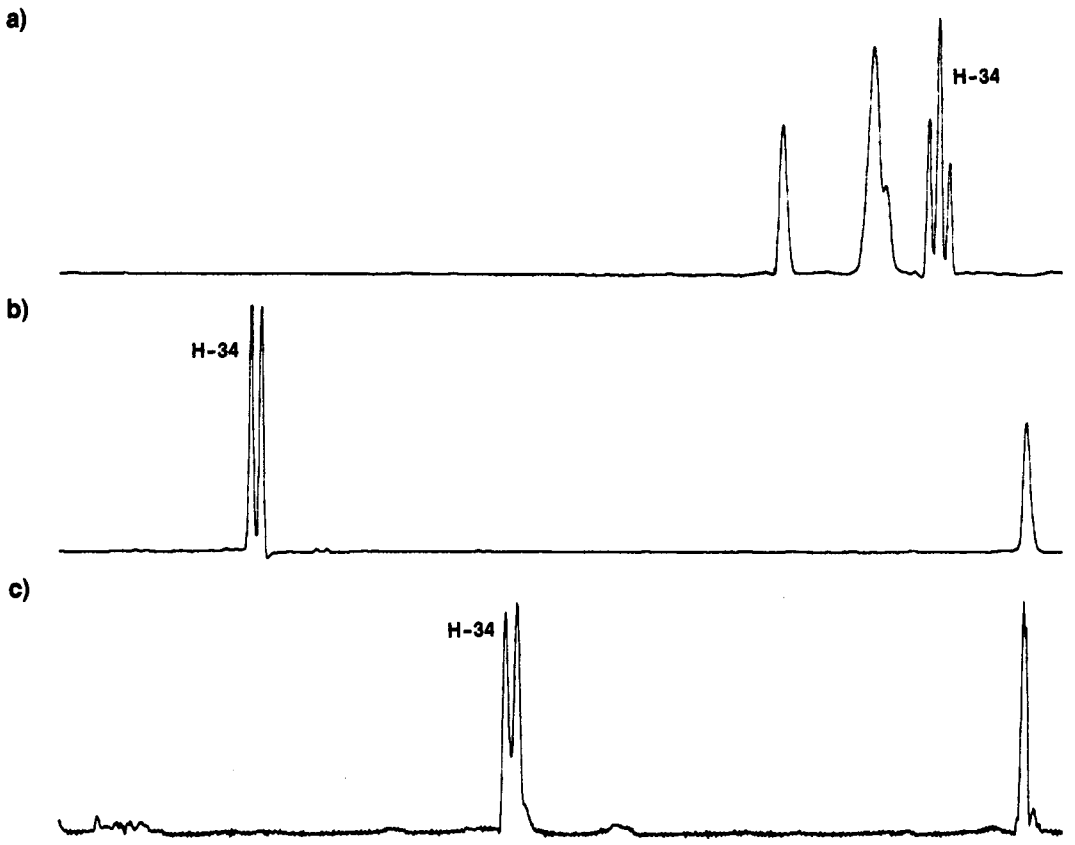

Figure 3. 500-MHz proton 1D NMR spectrum in $CDCl_3$ of H-34 in (a) 20-*epi*-bryostatin 3 (**2**), δ 5.85, (b) bryostatin 3 (**1**), δ 6.08, and (c) bryostatin 1 (**3**), δ 6.00.

Table III. 500-MHz ROESY Buildup Data Supporting Structures for Bryostatin 3 (1) and 20-*epi*-Bryostatin 3 (2) in $CDCl_3$ Including Comparisons with 1D NOE Data in CD_3CN

	Bryostatin 3 (1)				20-epi Bryostatin 3 (2)					
	proton–proton interaction	NOE calcd distance[a]	model measured distance[b]	NOE expl rate[c]	calcd rate[d]	NOE calcd distance[a]	model measd distance[b]	NOE expl rate[c]	calcd rate[d]	1D NOE enhancement[e]
(a)	20–22	3.5	4.2	17	5.7	2.67	3.3	86	24	3.4, 2.7[f]
	20–34	2.46	2.7	142	81	*g*	3.3	*g*	24	*h*
	22–23[i]	–	3.1	–	34	–	3.1	–	37	1.6, –[f]
	23–19 OH	2.37	2.5[j]	169.3	124	2.87	2.5[j]	58.8	133	2.4
(b)	4 CH_2	–	1.8	704[k]	–	–	1.8	809[k]	–	
	6 CH_2	–	1.8	878[k]	–	–	1.8	874[k]	–	
	10 CH_2	–	1.8	971[k]	–	–	1.8	936[k]	–	
	14 CH_2	–	1.8	881[k]	–	–	1.8	1200[k]	–	
	24 CH_2	–	1.8	1016[k]	–	–	1.8	979[k]	–	
	avg CH_2	–	1.8	890	–	–	1.8	960	–	
(c)	5–7	2.25	2.6	234	101	2.31	2.6	217	101	
	6e–5	2.36	2.5	176	128	2.44	2.5	154	128	
	6e–7	2.25	2.4	237	163	2.36	2.4	187	163	
	6a–28	3.22	2.9	40	76[l]	2.76	2.9	109	76[l]	
	7–28	3.22	3.3	40	36[l]	3.32	3.3	23	36[l]	
	7–29	2.53	2.6	186	150[l]	2.43	2.6	149	150[l]	

[a] See text. [b] In Å from a Drieding Model. [c] Initial buildup rates in s^{-1}. [d] In s^{-1} from Drieding distances. [e] % enhancement = integrated intensity of NOE enhancement/integrated intensity of the saturated peak in NOE difference spectra (in CD_3CN). [f] Reverse irradiation, i.e., 22/20, 23/22. [g] The close chemical shift $\Delta\delta$ = 0.02 ppm precluded measurement. [h] H20 and H34 are only 0.07 ppp apart and therefore too close to provide reliable 1D NOE measurement. [i] A cis geometry predicts a HH distance of 2.1 Å and therefore a strong positive NOE buildup, approximately 356 for **1**, 381 for **2**. [j] Distance from H23 to 19-oxygen; see text for discussion of H-bonding effect on H23–19OH measured distances. [k] Each rate is an average of the rates from the two cross peaks of a geminal pair in a CH_2 group. [l] Volume corrected for CH groups[7]. See text. Assumes a chair conformation for the six-membered ring.

elucidation. This was the source of the C-20 stereochemical error reported earlier.[1,2]

Experimental Section

NMR assignments were made at 500 MHz using the following strategy: (1) integrated proton areas from 1D spectra were assembled by TOCSY connectivities into *J*-coupled groups, (2) these groups were reassembled by COSY connectivities into adjacent groups, (3) resolution enhanced (line broadening = –1.63; Gaussian apodization = 0.8) 1D spectra and a knowledge from (1) and (2) provided *J*-coupling data, (4) HMQC data and the assigned proton data provided the carbon assignments for protonated carbons, (5) HMBC (8 and 4 Hz) data provided assignments for quaternary carbons, and (6) NOE data and *J* coupling data were used to determine stereochemistry.

ROESY was the technique of choice in molecules of this size for the following reasons: (1) numerous proton spectral overlaps

even at 500 MHz reduce the use of 1D kinetic NOE experiments, (2) because $\omega\tau_c \approx 1$, many NOESY cross peaks would be expected to be small or lost entirely, (3) spin diffusion is not as significant in molecules for which τ_c is short, and (4) relatively long relaxation times allow for longer linear ROESY buildup observation. The ROESY buildup rates led to more reliable cross-peak volumes, and thus internuclear proton–proton distances because nonlinear buildup rates and peaks opposite in sign to the diagonal ruled out false NOE interactions.

ROESY spectra were obtained nonspinning at 27 °C in $CDCl_3$ solution using a Kessler spin lock of 30° pulses, States–Haberkorn phase cycling, and arraying the mixing time of the spin lock over the range 50–175 ms. Homospoil irradiation and a recycle delay of 3.4–5 s were employed. From three to six mixing times were used in each experiment. Estimations of initial buildup rates of individual cross peaks were made by volume integration of cross peaks (the integral scale of each spectrum set to a consistent, arbitrary value), and by linear least-squares fit of the volumes versus mixing time to a straight line. Calculation of proton–proton distances were then made under the assumption that the initial buildup rate was proportional to r_{ab}^{-6} where r_{ab} is the proton–proton internuclear distance; a fundamental distance reference was obtained by assigning the geminal CH_2 proton–proton distance for all aliphatic CH_2 pairs in the molecule to 1.8 Å.

1D NOE's were obtained in acetonitrile solution by interleaving the collection of spectra in which either an off-resonance or on-resonance presaturation with $\gamma H_2 \approx 40$ Hz for 3 s was followed with an observed 90° pulse (total recycle delay = 7 s); difference spectroscopy then provided the results.

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Supplementary Material Available: Complete tables of ^1H (500-MHz) and ^{13}C (125-MHz) NMR assignments in $CDCl_3$ and IR, UV, and HR FABMS data for bryostatin 3 (1) and 20-*epi*-bryostatin 3 (2) (5 pages). This material is contained in many 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.

New Analogs of *cyclo*(Prn-Prn): Synthesis of Unsymmetric Octahydro-1*H*,5*H*-dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-5,10-diones

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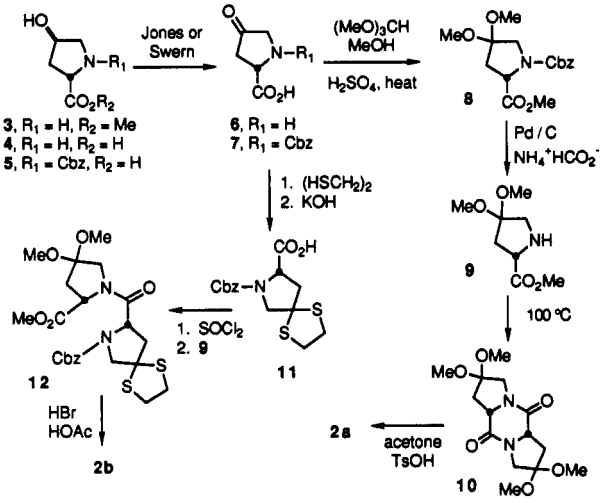
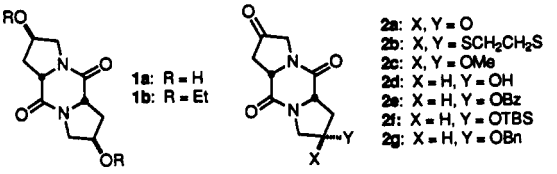
Synthesis of *trans*-4-hydroxy-L-proline cyclic dimer (**1a**, *cyclo*(Hyp-Hyp)) was first achieved by Kapfhammer and Matthes in 1933 and again in the 1970's by three other groups.[2-6] The only published derivatives of **1a** are *O*-ethyl ether **1b** and 4-oxo-L-proline cyclic dimer **2a** (*cyclo*(Prn-Prn)).[6-9] The highly symmetric, highly functionalized **2a** is an inviting framework for constructing conformationally restricted analogs of potential biological interest. In addition to a new preparation of **2a**, we report synthetic routes effectively differentiating the homotopic carbonyls and allowing for selective synthesis of congeners masking the C_2 symmetry.

Scheme I





Initially, we anticipated that **2a** would provide us with a readily available base of operations for our proposed derivatizations. Although ample supplies of **1a** were available by dimerization of *trans*-4-hydroxy-L-proline methyl ester (**3**),[2] we were unable to consistently reproduce the yield reported for DMSO/DCC oxidation to **2a**.[6] Furthermore, the long reaction time (5 days), low solubility of **2a** in most organic solvents (1 g/2000 mL of acetone), and the "extensive purification" required with this method discouraged our plans for scale up. A variety of other methods investigated for **1a** → **2a** conversion including DMSO/$(COCl)_2$,[10] DMSO/TFAA, DMSO/SO_3/pyr, DMSO/DCC/TFA/pyr, Jones reagent,[11] CrO_3/IRA400/DMF,[12] PCC/DMF, PCC/Al_2O_3,[13] and RuO_4[14-16] were

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