Isolation and Structure/Activity Features of Halomon-Related Antitumor Monoterpenes from the Red Alga *Portieria hornemannii*

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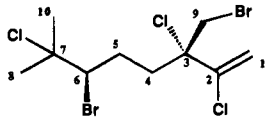
Ten halogenated monoterpenes (**2-6** and **8-12**) related to the novel antitumor compound halomon (**1**) or to the carbocyclic analog **7** have been isolated from different geographic collections of the red alga, *Portieria hornemannii*. Structures were assigned on the basis of spectral analyses (primarily NMR and MS). The absolute configuration of isohalomon (**2**) was further established by X-ray crystallography. The compounds were comparatively evaluated alongside **1** and **7** in the U.S. National Cancer Institute's *in vitro* human tumor cell line screening panel. The results provide some interesting initial insights into the structure/activity relationships in this series.

Introduction

A previous paper[1] described the isolation, structure elucidation, and novel National Cancer Institute (NCI) *in vitro* antitumor screening profile of 6(*R*)-bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene (halomon, **1**, NSC 650893) from extracts of the red alga *Portieria hornemannii* (Lyngbye) P. C. Silva. Subsequently, the NCI Decision Network Committee selected **1** for preclinical drug development. However, further research and development of **1** has been severely constrained by the very small amounts of natural **1** that have been available to date and the lack of synthetic access to **1**. Nevertheless, the limited, preliminary *in vivo* evaluations of **1** have been encouraging; daily ×5 doses of 50 mg/kg of **1** in an ip/ip xenograft model with the highly aggressive U251 brain tumor line have shown a high percentage, e.g., 40%, of apparent "cures" (R. Shoemaker, et al., NCI, unpublished). In the course of attempting to reisolate from various recollections of *P. hornemanniii* the gram quantities of **1** required for detailed *in vivo* xenograft testing, we encountered a series of related linear monoterpenes and carbocyclic analogs. Herein we report the isolation, structure elucidation, and comparative *in vitro* antitumor activity profiles for this series of compounds from *P. hornemannii* collected in the Philippines and Hawaii.

Results and Discussion

Chemistry. A recollection (April 1, 1992) of *P. hornemannii* from the original collection site[1] at Chanaryan in the Philippines provided 29 g of crude organic extract. Solvent–solvent partitioning of a portion of the extract between hexane and 10% aqueous methanol gave a monoterpene-rich hexane fraction, which was permeated through Bio-Beads S-X4. After removal of most of the halomon (**1**) by crystallization, normal-phase HPLC on silica gel yielded additional quantities of halomon and five other monoterpenes (**2**–**6**).



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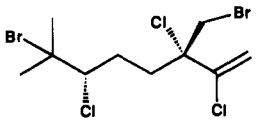

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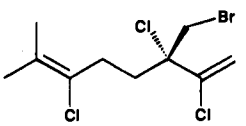

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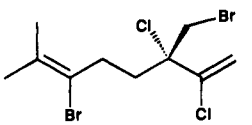

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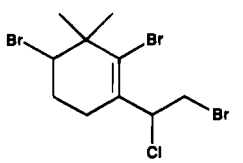

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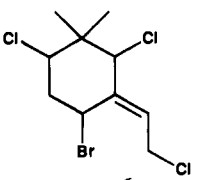

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Isohalomon (**2**) eluted as a shoulder on the backside of the halomon peak and was purified by repeated peak-shaving and recycling. Mass spectral analyses indicated that **2** was isomeric with halomon. Subtle chemical shift differences between **1** and **2** in the ^{1}H-NMR spectrum and more distinct ^{13}C-NMR differences at C6 and C7 led us to conclude that placement of the halogen substituents at C6 and C7 in **1** was reversed in **2**. These two compounds had been characterized as a mixture by Burreson *et al.*[2] nearly two decades ago. Our hypothesis was confirmed by X-ray diffraction analysis. A computer-generated perspective drawing of the final

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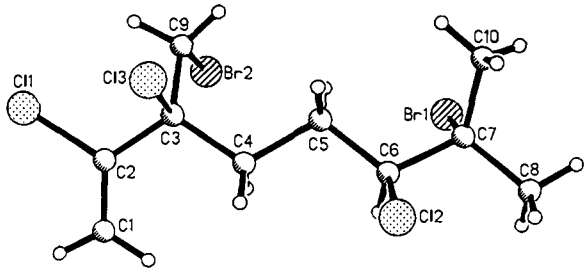


Figure 1. A computer-generated perspective drawing of isohalomon (**2**) showing all atoms. The absolute configuration was set by the anomalous scattering of the halogens.

X-ray model of isohalomon (**2**) is given in Figure 1, and the absolute configuration, set by the anomalous scattering of the halogen atoms, is as shown (3*S*,6*S*). All of the sp^3 centers in isohalomon, the chain from C3 to C8, are arranged in an extended conformation. The most interesting feature of isohalomon is its relation to halomon. The two structures have the same carbon skeleton and substitution pattern. They have the same absolute configuration at the chlorine-bearing center at C3, and they differ in the halogen atoms located at C6 and C7 as well as in the absolute configuration at C6. The relationship between halomon and isohalomon is formally a diatropic rearrangement of the halogen substituents at C6 and C7, and it is significant that in the X-ray structures of both compounds, the halogens at C6 and C7 have an antiperiplanar relationship. However, no isohalomon could be detected when halomon was heated in toluene (80 °C, 2 h).

Compounds **3** and **4** eluted prior to halomon in the HPLC analysis and presented similar ^{1}H NMR spectra (see Table 1). Notable features were the absence of the H6 resonance of **1** and **2** and downfield shifts of the methyl groups attached to C7. Mass spectral analyses indicated an additional site of unsaturation compared to **1** and **2**, establishing molecular formulas of $C_{10}H_{14}BrCl_3$ and $C_{10}H_{14}Br_2Cl_2$ for **3** and **4**, respectively. Thus, **3** is a dehydrobromo derivative of **2** and **4** is a dehydrochloro derivative of **1**. The stereochemistry at C3 is arbitrarily assigned as 3*S* on biogenetic grounds.

The molecular formula of **5** was defined as $C_{10}H_{14}Br_3Cl$ by HREIMS. Since only two olefinic carbons were observed in the ^{13}C-NMR spectrum, **5** had to be carbocyclic. COSY spectra provided two small spin systems, which were connected through HMQC/HMBC experiments. Placement of the halogens followed from ^{13}C-NMR chemical shifts (see Table 2). Gerwick had reported this structure, without stereochemistry, a decade ago,[3] but comparison of the two sets of NMR data indicated that the compounds must be diastereomers. Neither absolute nor relative configurations have been determined. The structure of compound **6**, $C_{10}H_{14}BrCl_3$, was elucidated in a similar manner and shown to be an HBr adduct of **7**, a compound which we had reported earlier from another collection of *P. hornemannii*.[1]

An earlier recollection of *P. hornemannii* (November 1991) from Chanaryan contained no significant (isolable) quantities of compounds **1**–**6** but instead contained a single linear monoterpene as its major constituent. The ^{1}H-NMR spectrum revealed resonances for three olefinic protons but otherwise resembled the spectra of **3** and **4**. Again, mass spectrometry provided the molecular formula, $C_{10}H_{15}BrCl_2$. These data constituted the necessary evidence for structure **8**, wherein

Table 1. ^{1}H-NMR Assignments for Compounds **2**–**6**, **8**, and **9**[a]

proton no.	2	3	4	5	6	8[b]	9
1	5.82 (d, 2.4)	5.80 (d, 2.4)	5.82 (d, 2.5)	3.62 (dd, 10.8, 5.4)	4.18 (ddd, 12.2, 0.3, 1)	5.76 (d, 2.4)	5.51 (dd, 17, 0.5)
	5.61 (d, 2.4)	5.59 (d, 2.4)	5.61 (d, 2.5)	3.49 (dd, 10.8, 10.3)	4.04 (dd, 12.2, 6.4)	5.57 (d, 2.4)	5.36 (dd, 10.8, 0.5)
2				5.41 (dd, 10.3, 5.4)	5.95 (dd, 9.3, 6.4)		5.94 (dd, 17, 10.8)
3							
4	2.68 (ddd)	2.32 (ddd)	2.34 (ddd)	2.50 (m)	4.97 (d, 4.9)	2.04 (m)	2.46 (ddd, 13.7, 11.5, 3.7)
	1.83 (ddd)	2.22 (ddd)	2.25 (ddd)	2.19 (m)			2.06 (ddd, 13.7, 11.6, 4)
5	2.57 (dddd)	2.58 (ddd)	2.70 (ddd)	2.32 (m)	2.67 (ddd, 15.1, 4.4, 2)	2.14 (m)	2.54 (dddd), 13.8, 11.5, 4, 1.7)
	2.07 (dddd)	2.50 (ddd)	2.62 (ddd)	2.20 (m)	2.53 (ddd, 15.1, 12.7, 4.9)		1.91 (dddd, 13.8, 11.6, 11.1, 3)
6	3.99 (dd, 10.7, 1.2)			4.32 (ddd)	4.84 (dd, 12.7, 4.4)	5.11 (dd, 7.3, 1.5)	4.06 (dd, 11.1, 1.7)
7							
8	1.76 (s)	1.75 (s)	1.76 (s)		4.38 (d, 1)	1.62 (s)	1.68 (s)
9	3.84 (d, 10.8)	3.82 (d, 10.8)	3.84 (d, 10.2)			3.82 (d, 10.8)	3.71 (d, 10.5)
	3.79 (d, 10.8)	3.76 (d, 10.8)	3.78 (d, 10.2)	1.34 (s)	1.01 (s)	3.78 (d, 10.8)	3.69 (d, 10.5)
10	1.91 (s)	1.80 (s)	1.83 (s)	1.41 (s)	1.29 (s)	1.68 (s)	1.80 (s)

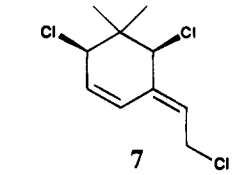
[a] Recorded at 500 MHz in $CDCl_3$; except as noted, assignments made via HMQC/HMQC experiments; data reported as δ (multiplicity, coupling constants in hertz). [b] Assigned by analogy.

Table 2. ^{13}C-NMR Assignments for Compounds **2**–**6**, **8**, and **9**[a]

carbon no.[b]	2	3	4	5	6	8	9
1	118.6	118.3	118.3	31.5	37.6	117.9	118.0
2	139.3	139.6	139.6	61.5	131.8	140.3	138.0
3	74.0	73.8	73.8	130.9	137.9	74.2	72.2
4	36.9	36.7	37.2	24.8	50.4	38.3	38.4
5	30.5	31.0	33.3	29.1	41.3	25.7	29.9
6	71.1	125.7	118.7	60.5	52.7	122.1	65.2
7	67.4	128.8	131.8	44.4	41.4	133.3	71.7
8	27.8	20.2	20.2	134.9	70.0	17.7	27.1
9	39.1	38.5	38.5	24.6	28.5	38.5	40.8
10	33.6	21.8	25.2	29.0	20.5	23.4	33.1

[a] Recorded at 125 MHz in $CDCl_3$; except as noted, assignments made via HMQC/HMBC experiments: data reported as δ. [b] Attached H denoted in Table 1.

the 6-halo substituents of **3** and **4** were replaced by hydrogen; this compound had been reported by Burreson *et al.*[2] from Hawaiian *P.* (formerly *Chondrococcus*) *hornemannii*.



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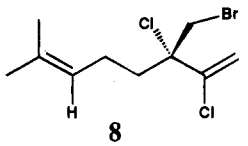

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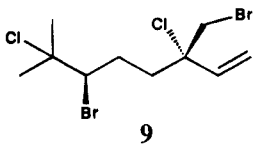

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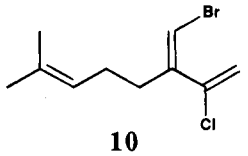

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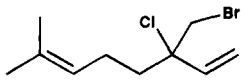

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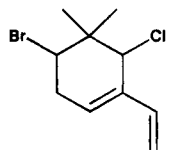

12

A third recollection (April 10, 1992) of *P. hornemannii* from Chanaryan provided a mixture of **1**–**5** and an additional major metabolite, **9**, $C_{10}H_{16}Br_2Cl_2$. The only noteworthy difference between halomon (**1**) and **9** lay in the olefinic region of the NMR spectra. Rather than signals for the 1,1-disubstituted olefin found in halomon, signals for an ABX system, representative of a monosubstituted carbon–carbon double bond, were observed in the ^{1}H-NMR spectrum of **9**. A series of COSY, HMQC and HMBC experiments confirmed that **9** was 2-dechlorohalomon.

P. hornemannii collected near the Halona Blowhole on the island of Oahu, HI, provided three additional known compounds, **10**,[4] **11**,[5] and **12**.[6] These compounds were identified by careful comparison of their spectral data with those published earlier. No stereochemical determinations were made.

Biology. The linear monoterpenes of this group comprised a series in which single sequential changes were present relative to adjacent members of the series (see Scheme 1). Thus, this relatively small group served as a substantial point of departure for structure/activity relationship insights.

Scheme 1

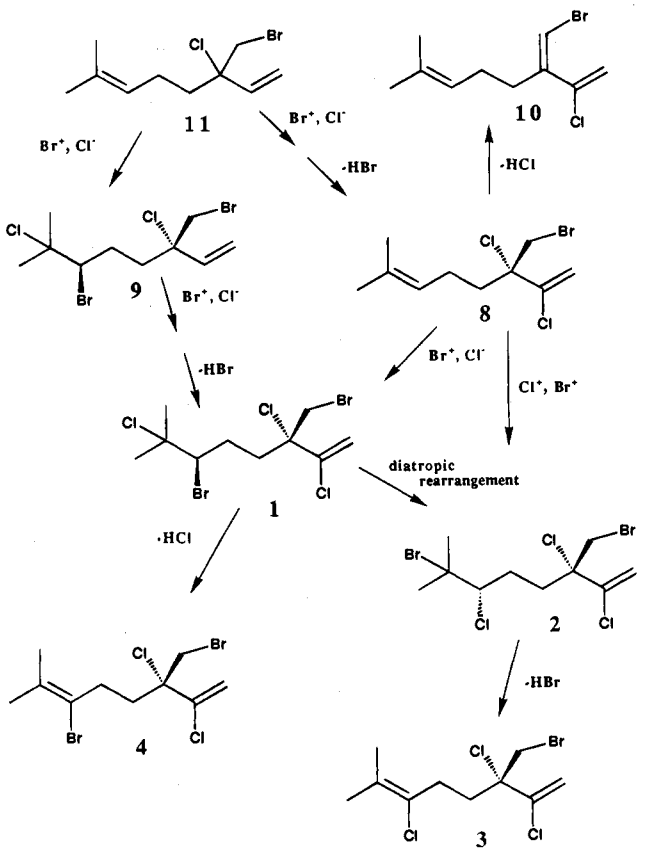

Table 3. Results of Comparative Testing of **1**–**12** in the NCI *in Vitro* Primary Antitumor Screen[10–12]

	mean panel response values ($\times 10^{-6}$ M)			Compare correlation coefficients[b]		
compd[a]	GI_{50}	TGI	LC_{50}	(GI_{50})	(TGI)	(LC_{50})
1	0.676	3.02	11.5	1.00	1.00	1.00
2	1.32	4.47	16.2	0.92	0.94	0.95
3	0.741	3.39	17.0	0.91	0.92	0.89
4	0.691	3.02	13.5	0.95	0.95	0.93
5	21.9	45.7	93.3	c	c	c
6	1.15	4.68	20.0	c	c	c
7	20.0	47.9	>100	c	c	c
8	47.0	>100	>100	c	c	c
9	26.1	77.0	>100	c	c	c
10	19.5	44.7	>100	c	c	c
11	33.1	>100	>100	c	c	c
12	>100	>100	>100	c	c	c

[a] Compounds **1**–**4** and **10** were tested[9] in quadruplicate; calculations are based upon the averaged values from all tests; in the replicate testing the standard errors averaged less than 10–15% of the respective means; due to limited supplies, compounds **6**–**9**, 11, and **12** were tested only once. [b] Compare correlation coefficients[11,13,14] were calculated using the GI_{50}-, TGI-, or LC_{50}-centered mean graphs of 1 (e.g., see ref 1) as the "seed" or benchmark compound for the comparisons against the corresponding GI_{50}, TGI, or LC_{50} mean graphs of the compounds of interest. [c] Correlation coefficient <0.5.

Compounds **1**–**4** uniformly exhibited the unique differential cytotoxicity profile reported earlier for halomon (**1**)[1] against the NCI panel of 60 human tumor cell lines, with comparable panel-averaged potency (Table 3). These results suggested that halogen at C7 was not essential to the activity and that the hybridization of C6 and C7 (sp^2 or sp^3) was not a critical factor. In contrast, compound **8** was relatively weakly cytotoxic, and the minimally differential activity profile showed no significant correlation to that of **1**, indicating that a

halogen at C6 was essential for the characteristic activity of **1**–**4**. Compounds **9**–**11** were also less potent than **1**–**4**, but comparable to **8** in both potency and lack of differential cytotoxicity. The lack of activity of **9** was of particular interest, indicating that halogen at C2 was required for "halomon-like" activity. As was observed previously with **7**,[1] the carbocyclic compounds (**5** and **12**) were considerably less cytotoxic than **1**–**4** and elicited no differential response from the tumor cell line panels. Compound **6** was more comparable in overall (panel-averaged) potency to halomon; however, there was little differential response of the cell lines, and consequently no significant correlation to the profile of **1**.

Recollection Efforts. It has long been known that slight geographic and/or temporal change can lead to considerable variation in the halogenated secondary metabolites produced by several genera of the Rhodophyta (red algae). After our initial attempt to obtain halomon from another, more readily accessible site in the Philippines failed, we succeeded in reisolating **1** from *P. hornemannii* collected at the original site (Chanaryan). We have subsequently embarked on an expanding search from that original site to survey populations of *P. hornemannii* for the presence of **1**. Further, we have begun monitoring the original site at Chanaryan; as noted above, we observed quite dramatic variation in monoterpene content over the course of just one month. These preliminary observations suggest that natural populations may not be consistently reliable sources of halomon adequate for preclinical development. Alternative sources, such as aquaculture and/or synthesis, need to be explored.

Experimental Section

Philippines *P. hornemannii*. The alga was collected at Chanaryan Island in November 1991 and April (1, 10, 19, 25) 1992 and frozen until extracted. The frozen alga was chopped into small pieces and then soaked in distilled water until thawed. The water was removed by centrifugation, followed by lyophilization of the aqueous extract. The algal pellet was extracted with CH_2Cl_2–MeOH (1:1, 3×) to give, after evaporation of solvent at 25 °C, the following yields of crude extract from each collection: November 1991, 8.9 g; April 1, 1992, 29.0 g; April 10, 1992,10.0 g; April 19, 1992, 21.0 g; April 25, 1992, 5.9 g.

Compounds **1**–**6**. The crude organic extract (9.5 g) from the Philippines collection of April 1, 1992, was suspended in 250 mL of MeOH–H_2O (9:1) and extracted with hexane (3 × 250 mL). The hexane extracts were evaporated and permeated through Bio-Beads S-X4 (8 × 60 cm) with hexane–CH_2Cl_2–EtOAc (2:4:1) to give five fractions differentiated by colored bands. Fraction 4 (1.05 g, light yellow band) was subjected to HPLC on silica (Rainin-Dynamax, 4.1 × 25 cm) with hexane (100 mL/min); six fractions were collected using UV detection at 215 nm and then identified as follows.

Halomon (**1**): Crystallization of fraction 5 from cold hexane and HPLC of the mother liquors (same conditions as above) yielded 430 mg of **1**, identical in all respects to that isolated earlier.[1]

Isohalomon (**2**): Peak shaving the trailing edge of the halomon peak provided 17 mg of **2**: $[\alpha]_D$ −25° (*c* 1.0, $CHCl_3$); HREIMS *m*/*z* 397.858 55 (calcd for $C_{10}H_{15}Br_2Cl_3$, 397.860 63).

3 and 4. Fraction 2 was rechromatographed as above to give **3** (18 mg), **4** (17 mg), and 20 mg of a mixture of the two. **3**: $[\alpha]_D$ −9.3° (c 1.2, $CHCl_3$); HREIMS *m*/*z* 317.9344 (calcd for $C_{10}H_{14}BrCl_3$, 317.93455) **4**; $[\alpha]_D$ −6.7° (c 1.0, $CHCl_3$); HREIMS *m*/*z* 361.8872 (calcd for $C_{10}H_{14}Br_2Cl_2$, 361.883 95).

5. Fraction 4 was recycled through the aforementioned HPLC system to provide **5**: $[\alpha]_D$ +125° (c 1.0, $CHCl_3$); HREIMS *m*/*z* 405.821 96 (calcd for $C_{10}H_{14}Br_3Cl$, 405.833 44).

6. Fraction 6 was subjected to HPLC (Rainin Dynamax-Cyano, 2.1 × 25 cm) with hexane (20 mL/min); the last fraction to elute was **6**: 20 mg; $[\alpha]_D$ +8.1° (*c* 5.2, $CHCl_3$); HREIMS *m*/*z* 317.941 50 (calcd for $C_{10}H_{14}BrCl_3$, 317.934 45).

8. An earlier collection of *P. hornemannii* (November, 1991) was processed in the same fashion. Prep-scale HPLC on silica as described above gave 8 mg of **8**: $[\alpha]_D$ −1.6° (*c* 3.0, $CHCl_3$); HREIMS *m*/*z* 283.9735 (calcd for $C_{10}H_{15}BrCl_2$, 283.9734).

9. A large collection of *P. hornemannii*, made on April 10 and 25, 1992, was processed as described above to give 660 mg of **9**: $[\alpha]_D$ +32.3° (*c* 1.19, $CHCl_3$); HREIMS *m*/*z* 363.8996 (calcd for $C_{10}H_{16}Br_2Cl_2$, 363.8987).

The ^{13}C- and ^1H-NMR data for **2-6** and **8-9** are provided in Tables 1 and 2.

Hawaiian *P. hornemannii*. The alga (319 g wet) was collected on May 29, 1992, near the Halona Blowhole, Oahu, by snorkeling. The alga was frozen and then freeze-dried at 30 °C. The volatiles were collected in a dry ice trap. The resulting emulsion was extracted with diethyl ether, which yielded a colorless oily residue (65 mg). HPLC (silica, hexane) furnished **12** (5 mg), identical with a degradation product of ochtodene, previously described by McConnell and Fenical.[6] Residual dry alga (80 g) was extracted with methylene chloride, which resulted in a green oily residue (460 mg). Sephadex LH-20 chromatography (MeOH-$CHCl_3$, 1:1) of that oil, followed by HPLC (silica, hexane) and then reversed-phase HPLC (C_{18}, MeCN), yielded **10** (35 mg) and 11 (5 mg). The structures of **10** and 11 were established by comparison with literature data.[4,5]

Single Crystal X-ray Analysis of Isohalomon (2). A single crystal roughly 0.2 × 0.2 × 0.3 mm was used for all measurements. Preliminary photographs showed orthorhombic symmetry. Accurate lattice constants of $a = 6.414(1)$, $b = 18.538(5)$, and c = 12.466(2) Å were determined from 25 diffractometer-measured 2θ values. Systematic absences, density considerations, and chirality were uniquely accommodated by space group $P2_12_12_1$, with one molecule of composition $C_{10}H_{15}Br_2Cl_3$ in the asymmetric unit. A total of 2303 diffraction maxima with $2\theta \leq 110°$ were collected using an ω:2θ scan technique with variable speed scans, and 1952 (85%) reflections were judged observed ($|F_o| \geq 4\sigma[F_o]$) and used in subsequent calculations. Lorentz, polarization and absorption corrections were applied to the data. Full-matrix least-squares refinements on F^2, using the SHELX93 package, converged to a final R of 6.37%. In the final model, positional and anisotropic thermal parameters were refined for all non-hydrogen atoms, and hydrogens were placed at ideal positions and allowed to refine using the riding model. The absolute configuration, shown in Figure 1, was determined using the Flack[7,8] method (final value 0.03(6) where 0.00 indicates the correct choice of absolute stereochemistry and 1.00 the enantiomeric choice).

Biological Testing and Data Analyses. Compounds were tested as described[9] in the NCI *in vitro* human tumor cell line screen.[10–12] Data calculations and analyses were performed as described.[11,13–14]

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Supplementary Material Available: Fractional coordinates, interatomic distances, interatomic angles, and thermal parameters (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.

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