Antineoplastic Agents. 362. Isolation and X-ray Crystal Structure of Dibromophakellstatin from the Indian Ocean Sponge *Phakellia mauritiana*[1]

George R. Pettit,*,† James McNulty,† Delbert L. Herald,† Dennis L. Doubek,† Jean-Charles Chapuis,† Jean M. Schmidt,† Larry P. Tackett,† and Michael R. Boyd‡

Cancer Research Institute, Department of Chemistry, Arizona State University, Tempe, Arizona 85287-2404, and Laboratory of Drug Discovery Research and Development, DTP, DCTDC, National Cancer Institute, Frederick Cancer Research and Development Center Frederick, Maryland 21702-1201

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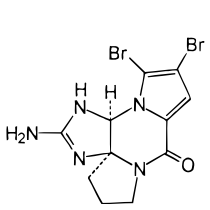
Bioassay-guided isolation procedures using human tumor cell lines led to isolation of dibromophakellstatin **(4)** from the Republic of Seychelles sponge *Phakellia mauritiana*. The isolation, X-ray crystal structure elucidation, absolute stereochemistry, and antineoplastic activity have been summarized. *P. mauritiana* was also found to contain dibromophakellin **(1)**, debromohymenialosine **(2)**, thymidine, deoxyuridine, and thymine.

A few of the more remarkable marine invertebrate nitrogen heterocyclic constituents such as manzamine dimer[2] and phorboxazoles A and B[3] have been found in the more rarely studied marine sponges such as the Indonesian *Prianos sp.*[2] and Western Australian *Phorbas sp.*[3] In general, however, marine porifera in the better known genera exemplified by *Agelas*,[4] *Axinella*,[5−8] *Dysidea*,[9,10] *Phakellia*,[11,12] *Theonella*,[13,14] and *Xestospongia*[15−17] have continued to yield the majority of new and biologically active nitrogen heterocyclic compounds.[18,19] Among the latter, more frequently encountered substances are dibromophakellin[12] (**1**), debromohymenialdisine[8] (**2**), and axinohydantoin[9] (**3**). The variety of pharmacological activities displayed by such metabolites has prompted further isolation and synthetic studies.[20−22]



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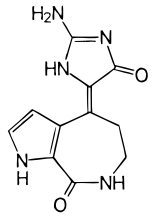

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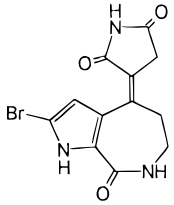

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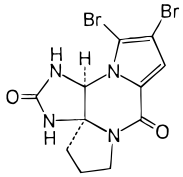

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In 1987, we collected the yellow-orange Indian Ocean sponge *Phakellia mauritiana* Dendy (170 kg wet weight) in the family Axinellidae (order Axinellida) at depths of 10−20 m in the Republic of Seychelles. The sponge was extracted with methanol−dichloromethane (200 L).

Table 1. Cell Growth Inhibitory Activity of Imidazoles **1**, **2**, and **4** against a Minipanel of Human Cancer Cell Lines (ED_{50}, μg/mL)

human cancer cell line	compd **1**	compd **2**	compd **4**
ovary, OVCAR-3	15.7	1.8	0.46
brain, SF-295	18.8	38.9	1.5
kidney, A498	17.8	24.7	0.21
lung, H460	22.0	42.0	0.62
colon, KM20L2	20.1	8.5	0.11
melanoma, SK-MEL-5	17.0	5.7	0.11

The methanol−water fraction was concentrated and extracted with ethyl acetate (4×) and further extracted with 1-butanol. Further solvent partitioning of the 1-butanol extract concentrated the cancer cell growth inhibitory activity into a 68-g methanol-soluble fraction that was further separated by gel permeation (Sephadex LH-20) chromatography in methanol. The antineoplastic activity was concentrated in the middle fractions, and from these the previously known dibromophakellin **(1)** and debromohymenialdisine **(2)** were readily identified. Human cancer cell line assays, however, showed that the fraction obtained between these two compounds possessed the most potent cell growth inhibitory activity. Thus, we undertook an extensive investigation of this fraction.

Chromatographic separation of the intermediate fraction on silica gel led to thymidine[23] and deoxyuridine.[24,25] Both have previously been isolated from the starfish *Acanthaster planci*,[26] and the corresponding uracils have been isolated from the ascidian *Aplidium fuscum*.[27] Further separation of the remaining, less polar fraction by Sephadex LH-20 partition chromatography gave a fraction that was used to obtain the new cancer cell growth inhibitor (Table 1) dibromophakellstatin **(4)** by crystallization. The mother liquors from the recrystallization were less active than the pure compound. Thymine was also isolated by recrystallization of another fraction from methanol.

By high-field (500 MHZ) ^{1}H-NMR spectroscopic analysis imidazolidinone **4** was found to be very similar to dibromophakellin **(1)**. However, comparison of the mass spectral data (**1**, $M^+ = 389$, **4**, $M^+ = 390$ *m/e*) and fragmentation showed important differences. The molecular formula $C_{11}H_{10}N_4Br_2O_2$ was deduced by HRFAB-MS analysis. The ^{13}C-NMR spectrum showed the pres-

* To whom correspondence should be addressed. This paper is dedicated to the memory of Dr. Matthew Suffness, who passed away in June, 1995.
† Arizona State University.
‡ National Cancer Institute.
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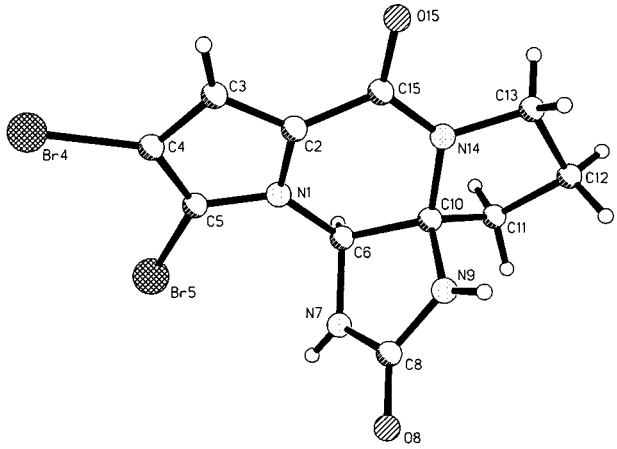

Figure 1. Computer-generated perspective view and X-ray numbering system for dibromophakellstatin (**4**).

ence of both an amide and a urea-type carbonyl group, and these facts suggested structure **4**, although isomeric structures also appeared possible.[28] Because of these uncertainties, an X-ray crystal structure determination was undertaken.

Crystals suitable for X-ray diffraction were obtained by slow evaporation of a methanol−toluene solution of dibromophakellstatin **(4)**. The crystal structure was readily solved and is shown in Figure 1. The absolute stereochemistry was easily determined due to the pronounced anomalous dispersion effects induced by the bromine atoms. SHELXL-93[29] refinement of the enantiomer shown in Figure 1 along with the Flack absolute structure parameter[30] resulted in a Flack parameter value of 0.03 (esd 0.04). The final standard residual *R* value was 0.0688, corresponding to a Sheldrick residual, $wR_2 = 0.1703$. Refinement of the mirror image of Figure 1 gave a Flack parameter value of 1.0001 (esd 0.0509). In addition, larger *R* values were observed for refinement of the mirror image e.g., $R = 0.0730$, $wR_2 = 0.1916$. As a consequence, the absolute stereochemistry for the two chiral centers of dibromophakellstatin **(4)** were assigned (using the X-ray numbering system) as 6*S*,10*R*.

Examination of the crystal packing of imadazolidinone **4** revealed an interesting feature that requires further comment. The intermolecular distances between the O8 atom of one molecule and the Br4 and Br5 atoms of adjacent, symmetry-related molecules are shorter than one might expect. These distances are 3.109 and 2.916 Å, respectively, as shown in Figure 2. If one uses the generally published values for the van der Waals radii for these two elements, i.e., 1.85 Å for Br and 1.50 Å for O, then a comparison of the sum of these radii (3.35 Å) with the above-observed X-ray determined distances would suggest that there must be a significant interaction (compression) occurring between the bromine atoms and the carbonyl oxygen O8 in the crystal packing of structure **4**. Such interactions are examples of what have been loosely termed "intermolecular nonbonded contacts," the most well-known example being the hydrogen bond. Numerous examples of contacts involving other atomic types have been observed and studied systematically.[31] As a result of these studies, a revision of the effective van der Waals radii for molecular crystals containing atoms such as F, S, Cl, Se, Br, and I has been proposed.[31c] For example, bromine, a "soft" atom (i.e., its nonbonded

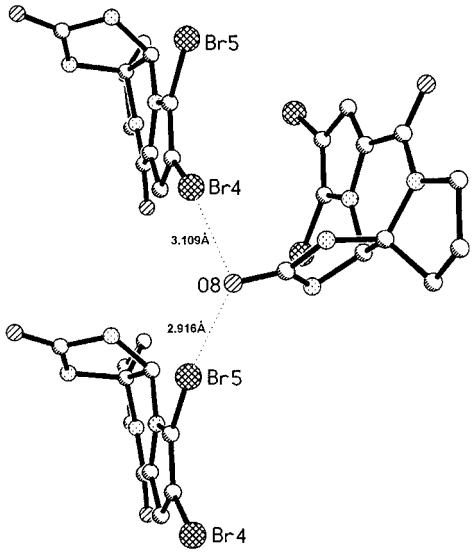

Figure 2. Adjacent, symmetry-related molecules of **4**, showing the non-bonded intermolecular contact interactions between bromine atoms and O8.

electrons are easily polarizable) undergoes "polar flattening", and the nonbonding electrons adopt a nonspherical shape. In effect, this results in a shorter van der Waals radius along the Br−C bond vector (1.54 Å) and a longer radius (1.84 Å) perpendicular to the Br−C bond. Oxygen (a "hard" atom), on the other hand, exhibits no spherical asymmetry in its nonbonding electron shape. The van der Waals radius remains fixed at 1.54 Å, regardless of whether there is a "head-on" or "sideways-on" contact with its intermolecular neighboring atom. As can be seen in Figure 2, because the contact between O8 and the bromine atoms in the crystal packing structure (**4**) is of the "head-on" type of interaction, the sum of the effective radii should more accurately be calculated to be 3.08 Å. As a consequence, the nonbonded interaction is less pronounced than initially assumed, being near the outer limits of a nonbonded contact. Finally, it should also be mentioned that an additional stabilizing factor may account for the close proximity of O8 and the bromine atoms, this being a normal H-bond between N9 on one molecule and O15 on an adjacent molecule of **4** (N···O distance, 2.821 Å). This has the effect of pulling adjacent molecules closer to one another, presumably tending to compress O8 into the Br atoms and possibly explaining the intermolecular nonbonded interaction observed.

Table 1 summarizes the cancer cell growth inhibitory results from an initial comparative evaluation of imidazoles **1**, **2**, and **4**. Dibromophakellstatin (**4**) appears to be the principal antineoplastic constituent present in the methanol extract of *P. mauritiana*. Dibromophakellstatin was further investigated in the U.S. National Cancer Institutes (NCI) full 60 cell-line human tumor panel. Approximately one-third of the panel lines showed GI_{50}-level growth inhibition with submicromolar concentrations of imadazolidinone **4**. Methodological details are referenced in the Experimental Section.

In summary, dibromophakellstatin **(4)** has been identified as the most significant cancer cell growth inhibitory substance in the 1-butanol extract of *P. mauritiana*. Currently, we are further evaluating the antineoplastic potential of this new porifera constituent. Also, the

isolation and structure elucidation of other biologically active constituents of *P. mauritiana* is in progress.

Experimental Section

General Methods. Solvents used for chromatographic procedures were redistilled. Sephadex LH-20 (25−100 μm) employed for gel permeation and partition chromatography was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Gilson FC-220 and FC-202 fraction collectors were used for chromatographic fractionation experiments. Silica gel GF Uniplates for TLC and silica gel Si 60 (230−400 mesh) were employed for chromatographic separation. All TLC plates were visualized under UV light (254 nm) and developed with anisaldehyde solution. Uncorrected melting points were determined on a digital Electrothermal apparatus. Mass spectra were obtained using a Kratos MS-50 spectrometer (70 eV). The NMR experiments were performed with a Varian VXR-500 instrument in the indicated solvent. Coupling constants (J) are given in Hz. These spectral techniques and direct comparisons with authentic samples were employed to identify previously known compounds. The X-ray crystallographic experiments were conducted with an Enraf-Nonius CAD-4 diffractometer.

Animal Collection and Extraction. *P. mauritiana* Dendy (170 kg wet wt) was collected near the island of Curieuse in the Republic of Seychelles from March to June 1987 at a depth of 10−20 m. The sponge was extracted twice with methanol−dichloromethane (1:1) (200 L per extraction). Thirty percent water was added to each extract, separating a dichloromethane phase that was concentrated using a Buchi R-150 class rotary evaporator.

Solvent Partition Sequence. The combined methanol−water fraction was concentrated by evaporation to 60 L of water and extracted (4×) with an equal volume of ethyl acetate followed by extraction with an equal volume of 1-butanol (4×). The butanol fraction (1313 g) was partitioned between water and hexane (5×), and then the water phase was partitioned between dichloromethane and water, yielding a dichloromethane fraction, of which 68 g was soluble in methanol. The methanol solution was chromatographed on a column of Sephadex LH-20 and eluted with methanol. The active, central fractions were further partitioned by column chromatography. A 31 g sample from the initial active fractions was separated by elution from a silica gel column with 9:1 dichloromethane−methanol to yield (following recrystallization from benzene−methanol) 1.1 g (6.5×10^{-4}%) of *dibromophakellin* (**1**). The last to elute LH-20-active fraction (3.1 g) was recrystallized from methanol to give 1.5 g (8.8×10^{-4}%) of *debromohymenialdisine* (**2**). An intermediate active fraction (3.7 g) from the LH-20 column was further separated using silica gel column chromatography and elution with 93:7 dichloromethane−methanol to provide three major fractions weighing 425, 95, and 39 mg. Another column chromatographic (9:1 ethyl acetate−hexane as eluent) separation of the 95 mg fraction on silica gel led to 29 mg (1.7×10^{-5}%) of *thymidine*, while analogous (93:2 dichloromethane−methanol as eluent) separation of the 39-mg fraction allowed 17 mg (1.0×10^{-5}%) of *deoxyuridine* to be isolated. A partition chromatographic separation of the 425-mg fraction on Sephadex LH-20 using 3:1:1 hexane−toluene−methanol afforded 12 mg (7.1×10^{-6}%, following recrystallization from methanol) of *thymine* and 31 mg (1.8×10^{-5}%, from a toluene−methanol recrystallization) of dibromophakellstatin (**4**): colorless crystals mp 245 °C dec; R_f 0.50 (CH_2Cl_2:CH_3OH 9:1); ^{1}H-NMR (DMSO) 8.25 (1H, brs), 7.97 (1H, brs), 6.92 (1H, s), 5.98 (1H, d, J = 2.4), 3.56 (1H, m), 3.44 (1H, m), 2.29 (1H, m), 2.09 (2H, m), 1.98 (1H, m); ^{13}C-NMR (DMSO) 157.8, 154.0, 125.3, 113.7, 105.5, 101.0, 78.9, 68.6, 44.0, 18.8, C-11 was hidden by the DMSO signal; MS (m/z rel) 392 (11), 390 (23), 388 (12), 318 (5), 311 (4), 309 (4), 252 (9), 187 (5), 139 (100), 111 (43), 82 (62), 80 (64); HRFAB-MS calcd for $C_{11}H_{11}N_4{}^{79}Br_2O_2$ $[M+H]^+$ 388.9248, found 388.9239.

X-ray Crystal Structure Determination of Dibromophakellstatin (4). A number of large, perfectly-formed, colorless, prismatic crystals of the compound were obtained by slow evaporation of a methanol−toluene solution. The best specimen, a large crystal, was cleaved to the approximate dimensions of 0.08 × 0.30 × 0.36 mm and mounted on the tip of a glass capillary tube with Super Glue. Data collection was performed at 26 ± 1° for a monoclinic system. All reflections corresponding to a complete quadrant, with $2\theta \leq 130°$, were measured using the $\omega/2\theta$ scan technique. Friedel reflections were also recorded, whenever possible, following each reflection. Subsequent statistical analysis of the complete reflection data set using the XPREP[32] program, coupled with density measurements, indicated the space group was $P2_1$, the asymmetric unit of the cell containing a single molecule of dibromophakellstatin **(4)**.

Crystal data: $C_{11}H_{10}Br_2N_4O_2$, monoclinic space group $P2_1$, with a = 8.039(1) Å, b = 6.982(1) Å, c = 11.435(2) Å, β = 97.342(21)°, V = 636.6(7) Å^3, λ (Cu Kα) = 1.541 84 Å, ρ_c = 2.035 g cm^{-3} for Z = 2 and F W = 390.05, F(000) = 380. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 2110 unique reflections (R(int) = 0.0587) remained, of which 2102 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of ψ-scans).[33] Structure determination was readily accomplished with the direct methods program SIR92.[34] All non-hydrogen coordinates were located in a routine run using default values in that program. The remaining hydrogen atoms, calculated at optimum positions for imidazole **4** using the program SHELXL-93, were assigned thermal parameters equal to 1.5 times the Uiso value of the atom to which they were attached, and then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process with the SHELXL-93 software package. The final standard residual R value for the dibromo model was 0.0688 for observed data (2102 reflections) and 0.0708 for all data (2110 reflections). The corresponding Sheldrick R values were wR_2 of 0.1703 and 0.1709, respectively. A final difference Fourier map showed significant residual electron density, the largest difference peak and hole being 1.671 and −1.289 e/Å^3, respectively. However, a majority of this residual

density was within close proximity of the two bromine atoms and consequently was attributed to the bromine atoms themselves. Final bond distances and angles were within acceptable limits. The final structure, depicting the absolute stereochemistry of dibromophakellstatin **4**, is shown in Figure 1.[35]

Biological Studies. The initial comparative studies of human tumor growth inhibition by imidazoles **1**, **2**, and **4** were performed using the sulforhodamine B (SRB) assay.[36] The evaluation of imidazolidinone **4** in the NCI 60 cell-line human tumor panel [37] was performed as described.[38] Data analyses and response parameter definitions were previously summarized.[39] NCI panel cell lines showing GI_{50}-level growth inhibition with submicromolar concentrations of **4** were as follows (the *negative* $\log_{10}$ GI_{50} values are shown in parentheses and represent averages of quadruplicate determinations): leukemia, K-562 (6.16); lung, A549 (6.17), HOP-92 (6.31), H226 (6.06), H460 (6.08), H522 (6.62); colon, COLO205 (6.10), HCT116 (6.24), HT29 (6.2); brain, SF539 (6.21), SNB75 (6.11), U251 (6.23); ovary, OVCAR5 (6.17), OVCAR8 (6.92); kidney, 786−0 (6.22), A498 (6.89); prostate, PC-3 (6.15); breast, MCF7/ADR-RES (6.28), MDA-MB-231 (6.14), HS578T (6.06).

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References and Notes

(1) Antineoplastic Agents. Part 361: McGown, A. T.; Jayson, G.; Pettit, G. R.; Haran, M. S.; Ward, T. H.; Crowther, D. Manuscript in preparation.
(2) Ohtani, I. I.; Ichiba, T.; Isobe, M.; Kelly-Borges, M.; Scheuer, P. J. *J. Am. Chem. Soc.* **1995**, *117*, 10743−10744.
(3) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126−8131.
(4) Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1995**, *36*, 7893−7896.
(5) Miller, S. L.; Tinto, W. F.; Yang, J-P.; McLean, S.; Reynolds, W. F. *Tetrahedron Lett.* **1995**, *36*, 5851−5852.
(6) Pettit, G. R.; Gao, F.; Schmidt, J. M.; Chapuis, J-C.; Cerny, R. L. *BioMed. Chem. Lett.* **1994**, *4*, 2935−2940.
(7) Pettit, G. R.; Herald, C. L.; Leet, J. E.; Gupta, R.; Schaufelberger, D. E.; Bates, R. B.; Clewlow, P. J.; Doubek, D. L.; Manfredi, K. P.; Rützler, K.; Schmidt, J. M.; Tackett, L. P.; Ward, F. B.; Bruck, M.; Camou, F. *Can. J. Chem.* **1990**, *68*, 1621−1624.
(8) Cimino, G.; De Rosa, S.; De Stefano, S.; Mazzarella, L.; Puliti, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 767−768.
(9) Clark, W. D.; Crews, P. *Tetrahedron Lett.* **1995**, *36*, 1185−1188.
(10) Kobayashi, M.; Wang, W.; Ohyabu, N.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1995**, *43*, 1598−1600.
(11) Pettit, G. R.; Xu, J-P.; Cichacz, Z. A.; Williams, M. D.; Chapuis, J-C.; Cerny, R. L. *BioMed. Chem. Lett.* **1994**, *4*, 2677−2682.
(12) Sharma, G.; Magdoff-Fairchild, B. *J. Org. Chem.* **1977**, *42*, 4118−4124.
(13) Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 1177−1181.
(14) Kobayashi, J.; Hagaki, F.; Shigemori, H.; Takao, T.; Shimonishi, Y. *Tetrahedron* **1995**, *51*, 2525−2532.
(15) Kobayashi, M.; Chen, Y-J.; Aoki, S.; In, Y.; Ishida, T.; Kitagawa, I. *Tetrahedron* **1995**, *51*, 3727.
(16) Kong, F.; Anderson, R. J. *Tetrahedron* **1995**, *51*, 2895−2906.
(17) Coval, S. J.; Conover, M. A.; Mierzwa, R.; King, A.; Puar, M. S.; Phife, D. W.; Pai, J.-K.; Burrier, R. E.; Ahn, H-S.; Boykow, G. C.; Patel, M.; Pomponi, S. A. *BioMed. Chem. Lett.* **1995**, *5*, 605.
(18) Faulkner, D. J. *Nat. Prod. Rep.* **1994**, *11*, 355−394.
(19) Pettit, G. R.; Hogan-Pierson, F.; Herald, C. L. *Anticancer Drugs from Animals, Plants, and Microorganisms*;, John Wiley-Interscience: New York, 1993.
(20) Foley, L. H.; Buchi, G. *J. Am. Chem. Soc.* **1982**, *104*, 1776−1777.
(21) Xu, Y.; Phan, G.; Yakushijin, K.; Horne, D. A. *Tetrahedron Lett.* **1994**, *35*, 351−354.
(22) Annoura, H.; Tatsuoka, T. *Tetrahedron Lett.* **1995**, *36*, 413−416.
(23) Tollin, P.; Wilson, H. R.; Young, D. W. *Nature* **1968**, *217*, 1148−1149.
(24) Dekker, C. A.; Todd, A. R. *Nature* **1950**, *166*, 557−558.
(25) Rahman, A.; Wilson, H. R. *Nature* **1971**, *232*, 333−334.
(26) Komori, T.; Sanechika, Y.; Ito, Y.; Matsuo, J.; Nohara, T.; Kawasaki, T.; Schulten, H. R. *Liebigs Ann. Chem.* **1980**, *668*, 653−824.
(27) Dematte, N.; Guerriero, A.; Lafargue, F.; Pietra, F. *Comp. Biochem. Physiol.* **1986**, *84B*, 11−13.
(28) Fedoreyev, S. A.; Utkina, N. K.; Ilyin, S. G.; Reshetnyak, M. V.; Maximov, O. B. *Tetrahedron Lett.* **1986**, *27*, 3177−3180.
(29) Sheldrick, G. M. *SHELXL93. Program for the Refinement of Crystal Structures*; University of Gottingen, Germany, 1993.
(30) Flack, H. D. *Acta. Crystallogr.* **1983**, *A39*, 876−881.
(31) (a) Rowland, R.; Allen, F.; Carson, W.; Bugg, C. In *Crystallographic and Modeling Methods in Molecular Design*; Bugg, C., Ealick, S., Eds.; Springer-Verlag: New York, 1990; pp 229−253. (b) Nyburg, S. C.; Faierman, C. H.; Prasad, L. *Acta Crystallogr.* **1987,** *B43*, 106−110. (c) Nyburg, S. C.; Faerman, C. H. *Acta Crystallogr.* **1985**, *B41*, 274−279. (d) Britton, D.; Dunitz, J. D. *Helv. Chim. Acta* **1980**, *63*, 1068−1073. (e) Rosenfield, E. E., Jr.; Parthasarathy, R.; Dunitz, J. D. *J. Am. Chem. Soc.* **1993**, *99*, 4860−4862.
(32) XPREP, The automatic space group determination program in the SHELXTL-PC program package.
(33) North, A. C.; Phillips, D. C.; Matthews, F. S. *Acta. Crystallogr.* **1969/8**, *A24*, 351−359.
(34) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M.; Polidori, G.; Camalli, M. *SIR92—A Program for Automatic Solution of Crystal Structures by Direct Methods*, Dipartimento Geomineralogico, University of Bari, Italy.
(35) Preparation of Figure 1 was done with "SHELXTL-PC Version 5.03, (1994)," an integrated software system for the determination of crystal structures from diffraction data, Siemens Industrial Automation, Madison, WI 53719.
(36) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(37) Boyd, M. R. In *Current Therapy in Oncology*; Niederhuber, J. E., Ed.; B. C. Becker, Inc.: Philadelphia, 1993; pp 11−12.
(38) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(39) Boyd, M. R.; Paull, K. D. *Drug Develop. Res.* **1995**, *34*, 91−109.
(40) X-ray data of compound **4** have been deposited at the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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