

Antineoplastic Agents. 380. Isolation and X-ray Crystal Structure Determination of Isoaaptamine from the Republic of Singapore *Hymeniacidon* sp. and Conversion to the Phosphate Prodrug Hystatin 1¹

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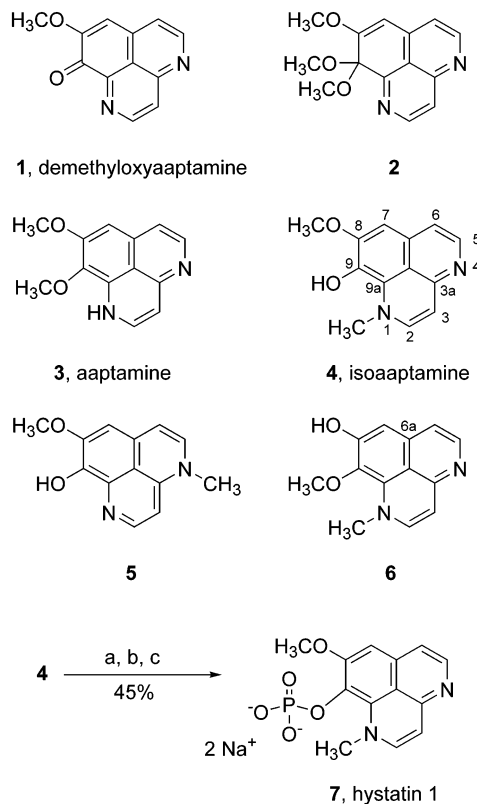
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By use of bioassay (murine P388 lymphocytic leukemia cell line) guided isolation procedures, extracts of the Republic of Singapore marine sponge *Hymeniacidon* sp. were found to contain demethoxyaaptamine (**1**) and aaptamine (**3**) as prominent cancer cell growth inhibitory constituents accompanied by the trace, albeit more active, component isoaaptamine (**4**). The isolation, X-ray structure elucidation, and antineoplastic and antimicrobial activities of isoaaptamine (**4**) have been summarized. Because of instability, isoaaptamine (**4**) was converted to a stable sodium phosphate prodrug designated hystatin 1 (**7**).

Marine Porifera continue to be a rapidly expanding source of new drug candidates^{2,3} with unprecedented structures^{4–6} and very important biological activities.^{7,8} From the onset⁶ of our investigations directed at exploring marine organisms as sources of new anticancer drug candidates, we have continued to evaluate marine sponge constituents.^{6–8} In 1992, during our marine invertebrate survey in the Republic of Singapore, we located a previously unknown species of sponge belonging to the genus *Hymeniacidon* (order Halichondrida) that yielded methanol–dichloromethane extracts active against the murine P388 lymphocytic leukemia cell line. A 500 kg (wet wt) recollection of the organism was employed to isolate the cancer cell line inhibitory components utilizing the P388 cell line to guide the separations.

The sponge methanol–water and methanol–dichloromethane extracts (see Experimental Section) were partitioned between water and dichloromethane. The dichloromethane phase was partitioned between hexane and methanol–water (9:1) to give polar fraction A. The initial aqueous phase was next extracted with ethyl acetate followed by 1-butanol to yield alcohol-soluble fraction B (1.08 kg). A series of Sephadex LH-20 gel permeation and partition chromatographic steps were used to separate fraction A and led to the previously known demethoxyaaptamine (**1**)⁹ and the dimethyl ketal **2**, likely an artifact of the isolation process.⁹ Demethoxyaaptamine (**1**) was isolated in gram quantities and exhibited a P388 ED₅₀ of 0.31 μg/mL, while the much less abundant dimethyl ketal **2** was inactive (P388 ED₅₀ 59 μg/mL).

Fraction B was separated, and aaptamine (**3**)^{10a} was also isolated in gram quantities along with the minor (10^{–5}% yields) constituent isoaaptamine (**4**),¹¹ each as the hydrochloride salt. The cancer cell growth inhibitory activities of compounds **1**, **3**, and **4** are summarized in Table 1.



^a(BnO)₂(O)PH, *i*Pr₂EtN, DMAP, CCl₄, CH₃CN, -10 °C;

^b(CH₃)₃SiBr, CH₂Cl₂, rt; ^cNaOCH₃, CH₃OH, rt

The structures of compounds **1**, **2**, and **3** were readily deduced from spectral properties and literature comparisons.^{9,10a} However, the structure of compound **4** proved to be troublesome. Analysis of the ¹H and ¹³C NMR spectra indicated that it was nearly identical to isoaaptamine (**4**), which was first reported by Fedoreev.^{11a} Subsequently, it was again isolated by Kashman^{11b} and Shen.^{11c} In our experiments, all of the proton and carbon signals were shifted downfield slightly owing to its isolation as the

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[†] CRI. We dedicate this contribution to the memory of Dr. Harry B. Wood (July 30, 1919–August 7, 2002), an outstanding pioneer of anticancer drug development.

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Table 1. Comparative Cancer Cell Line Results (ED₅₀ µg/mL) for Demethyloxaaptamine (1), Aaptamine (3), Isoaaptamine (4), and Hystatin 1 (7)

cell type	cell line	demethyloxaaptamine (1)	aaptamine (3)	isoaaptamine (4)	hystatin 1 (7)
leukemia	murine P388	0.31	3.60	0.28	3.0
ovary	OVCAR-3	0.39	4.90	1.20	ND
CNS	SF-295	2.80	4.10	2.60	ND
renal	A498	4.20	3.20	2.20	ND
lung	NCI-H460	2.30	3.20	2.40	>10
colon	KM20L2	2.10	3.60	2.30	>10
melanoma	SK-MEL-5	1.00	4.30	1.60	ND

hydrochloride salt. The shift of one of the methyl carbon resonances from δ 60.7 in aaptamine (3) to 45.9 in isoaaptamine (4) suggested the likelihood that an *O*-methyl had shifted to an *N*-methyl position. Since isoquinoline structures 5 and 6 also seemed consistent with the NMR data, it was apparent that structure 4 could not initially be assigned with certainty. But scrutiny of the NMR NOESY and ROESY spectra did point to the likelihood of structure 4. As an example, a cross-peak in the ROESY spectrum between H-7 (δ 7.15) and the pyridine-like hydrogen H-6 (δ 6.80) was consistent with structure 4. NOESY cross-peaks between the proposed methoxy on C-8 (δ 4.03) and both H-7 and the phenolic hydroxy (δ 9.4) were commensurate with structure 4 or 5 and allowed us to rule out the methoxy analogue 6. On the basis of the NMR data, we were unable to rule out the possibility of structure 5 unambiguously. Because of possible long-range correlations that can occur through azaheterocycles, and in consideration of the important cancer cell line and antimicrobial activity of isoaaptamine (4), approaches to an unequivocal structure elucidation were undertaken based on X-ray crystal structure determination.

Since compound 4 was isolated as a yellow amorphous hydrochloride salt that resisted crystallization, other salt derivatives were explored. Changing the anion to perchlorate, for example, gave beautiful yellow-red needles. Unfortunately, these proved to be too thin for X-ray analysis. Other anions that were evaluated were even less promising. In view of the electron-rich structure of 4, a picric acid derivative was considered, since it might crystallize as both an acid-base and π - π complex. This proved to be the case. Single crystals of the picrate were formed by slow crystallization of a methanol-toluene solution of picric acid and isoaaptamine (4). The structure of the colorful π - π picrate complex verifies that our compound was indeed identical with structure 4. More recently, isoaaptamine (4) has been synthesized by Walz and Sundberg.¹²

One noteworthy feature of the crystal structure of the picrate was the very unique behavior exhibited by the toluene solvate. The molecule lies on the cell edges and is centered on an inversion center at 0.00, 0.00, 0.500. As a result, the toluene molecule was found to exist in two conformations, each of 1/2 occupancy and overlapping, inverted images of one another. The methyl substituent of one toluene molecule (e.g., CS4A of the molecule composed of ring atoms CS1, CS2, CS3, CS4, CS5, and CS2A) and the carbon atom at the *para*-ring position of the other toluene molecule (e.g., the ring consisting of CS1A, CS2A, CS3A, CS4A, CS5A, and CS2) both occupy the same site (within the resolution of the data), and as a consequence, both of these atoms were assigned to the same site.

For a variety of reasons, the isolation of isoaaptamine (4) as the most interesting cancer cell growth inhibitor of the Singapore sponge *Hymeniacidon* sp. is noteworthy. Aaptamine (3), as the parent alkaloid, was first isolated from the marine sponge *Aaptos aaptos*.^{10a} Later, it was isolated from several other species of sponges.^{10b} The

Table 2. Comparative Antimicrobial Activities of Isoaaptamine (4) and Hystatin 1 (7)

microorganism	range of minimum inhibitory concentration (ug/mL)	
	4	7
<i>Cryptococcus neoformans</i>	64	n.i.
<i>Candida albicans</i>	n.i. ^a	n.i.
<i>Staphylococcus aureus</i>	16–32	64
<i>Streptococcus pneumoniae</i>	8–32	16–32
<i>Enterococcus faecalis</i>	32–64	n.i.
<i>Micrococcus luteus</i>	32–64	n.i.
<i>Escherichia coli</i>	n.i.	n.i.
<i>Enterobacter cloacae</i>	n.i.	n.i.
<i>Stenotrophomonas maltophilia</i>	n.i.	n.i.
<i>Neisseria gonorrhoeae</i>	<0.5	<0.5

^a n.i.: no inhibition at 64 µg/mL.

discovery of the aaptamines in *Hymeniacidon* sp. suggests that unusual isoquinolines may enjoy a broader species and geographic distribution. Also, the large quantities of aaptamine (3) contained in *Hymeniacidon* sp. would suggest a relatively important biological role. Consequently, we have been proceeding with an extended study of the aaptamines, especially isoaaptamine (4).

When newly isolated, isoaaptamine (4) appears as a yellow-colored powder that rapidly changes to dark green and finally brown or black, presumably due to air oxidation. Because of this instability and resultant degradation, attempts at preparation of a variety of simple derivatives generally proved to be elusive. Hence, attention was directed at preparation of a stable prodrug that would retain the biological activity. For that purpose, isoaaptamine (4) was first phosphorylated¹³ with dibenzyl phosphite. Cleavage of the benzyl ester by means of trimethylsilyl bromide¹⁴ and reaction of the resulting phosphoric acid with sodium methoxide afforded the relatively more stable disodium phosphate prodrug designated hystatin 1 (7).

Biology. Evaluation of aaptamines 1, 3, 4, and 7 against our Institute's human tumor cell line panel (Table 1) and the NCI 60-cell line human tumor screen^{15–18} was undertaken. Isoaaptamine (4) had antifungal and antibacterial activities, and the prodrug hystatin 1 (7) retained some of the antibacterial activity (Table 2).

Experimental Section

General Experimental Procedures. Uncorrected melting points were determined on a digital Electrothermal apparatus. The NMR experiments were performed with a Varian VXR-500 instrument in the indicated solvent. Mass spectra were obtained using a Kratos MS-50 spectrometer (70 eV). The X-ray crystallographic experiments were conducted with an Enraf-Nonius CAD-4 diffractometer. 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 experi-

ments. Silica gel GF Uniplates were employed for monitoring the chromatographic separations. All TLC plates were visualized under UV light (254 nm) and developed with phosphomolybdic acid spray reagent.

Hymeniacion sp. (Halichondrida:Hymeniacionidae). The initial specimen of this bright orange encrusting-lobular sponge, a previously undescribed species, was collected on the north side of Terumbu Pemalang Besar Reef, Republic of Singapore, at a depth of 5–10 m in 1989. During 1992, 500 kg (wet wt) of the sponge was re-collected in this general area.

Animal Extraction and Solvent Partitioning. The methanol shipping solution was removed and extract concentrated to 100 L of an aqueous suspension, which was successively extracted with dichloromethane (6 × 50 L), ethyl acetate (6 × 50 L), and 1-butanol (4 × 50 L). The 1-butanol fraction was concentrated to dryness and partially redissolved in 2 L of methanol.

Isolation of the Aaptamines. The methanol-soluble portion (1084 g) of the 1-butanol fraction was fractionated by a gel permeation/partition sequence. The major component isolated was aaptamine (**3**, 8.8 g, $1.7 \times 10^{-3}\%$), having spectral properties in complete accord with the literature values. The minor but more active iso-aaptamine (**4**, 0.22 g, $4.4 \times 10^{-5}\%$) was obtained as an amorphous yellow powder: mp 200–205 °C (dec); ¹H NMR (DMSO) δ 3.96 (3H, s, OMe), 4.03 (3H, s, NMe), 6.25 (1H, d, $J = 7.2$, H-3), 6.80 (1H, d, $J = 7.3$, H-6), 7.15 (1H, s, H-7), 7.25 (1H, d, $J = 7.3$, H-5), 7.73 (1H, d, $J = 7.2$, H-2), 9.4 (1H, br s, OH), 12.7 (1H, br s, NH); ¹³C NMR (DMSO) δ 45.9 (NMe), 56.5 (OMe), 97.3 (C-3), 101.5 (C-7), 113.1 (C-6), 118.1 (C-9b), 127.7 (C-5), 129.3 (C-6a and C-9a), 132.2 (C-9), 148.9 (C-2), 149.2 (C-3a), 153.6 (C-8); MS m/z 228 (100), 213 (94), 185 (60), 170 (47), 155 (10), 142 (22), 127 (7), 115 (12), 84 (7); HREIMS m/z 228.0903 (calcd for C₁₃H₁₂N₂O₂, 228.0899).

Isoaaptamine Perchlorate. A sample of iso-aaptamine (**4**) (1.0 mg) was dissolved in methanol (1.0 mL) in a small crystallization tube and two drops of perchloric acid (70%) allowed to run down the side of the tube. Thin red needles were slowly deposited after standing at room temperature for several hours. The crystals proved to be too thin for X-ray analysis and were not further characterized due to decomposition on standing.

Isoaaptamine Picrate. Picric acid was washed with water, and the damp acid was dissolved in methanol at a concentration of about 1 M. To a 1.0 mg sample of iso-aaptamine (**4**) hydrochloride in a few drops of methanol was added 0.25 mL of the picric acid solution. The orange solution was heated to about 60 °C and diluted to 1.0 mL with toluene. The solution was filtered hot, allowed to cool to room temperature, and then placed in a freezer (approximately –20 °C), where red-colored single crystals (dp 95–97 °C) slowly formed over 72 h.

X-ray Crystal Structure Determination of Isoaaptamine (4**).** A few, marginal quality, red, rod-shaped crystals of the complex were obtained from toluene–methanol solution. The best specimen, $0.48 \times 0.10 \times 0.04$ mm, was mounted on the tip of a glass fiber with Superglue. Data collection was performed at $24 \pm 1^\circ$ for a monoclinic system. All reflections corresponding to a complete quadrant, with $2\theta \leq 130^\circ$, were measured using the $\omega/2\theta$ scan technique. Subsequent statistical analysis of the complete reflection data set using the XPREP¹⁹ program indicated the space group was $P2_1/c$, the asymmetric unit of the cell containing a single molecule each of the parent heterocyclic compound and picric acid, along with 1/2 a molecule of toluene. Crystal data: C₁₃H₁₂N₂O₂·C₆H₅N₃H₇·1/2C₇H₈, monoclinic space group $P2_1/c$, with $a = 7.983(1)$ Å, $b = 12.839(3)$ Å, $c = 21.522(5)$ Å, $\beta = 92.920(1)^\circ$, $V = 2203.0(8)$ Å³, $\lambda(\text{Cu K}\alpha) = 1.54184$ Å, $\rho_c = 1.518$ g cm⁻³ for $Z = 4$ and $fw = 503.43$, $F(000) = 1044$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 3141 unique reflections ($R(\text{int}) = 0.0505$) remained, of which 2353 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 psi-scans).²⁰ Structure determination was readily accomplished with the direct-methods program SIR92.²¹ All non-hydrogen atom coordinates (excluding toluene solvent atoms) were located in a structure solution run using the RANDOM option and allowing NREF to have the maximum value (499) for that program. The non-hydrogen solvent and the hydrogen atom positions on iso-aaptamine (**4**) and picric acid were determined from difference Fourier maps using the program SHELXL-93.²² The hydrogen atoms were assigned thermal parameters equal to 1.5 the U_{iso} 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 assignment of nitrogen atom positions in the aromatic ring of the heterocycle could not be made with any certainty based upon bond distance information alone. Consequently, N versus C occupancy refinements were made on the individual models produced by alternating the N atom over all possible suspected positions which might contain nitrogen. The N and C character of the various positions investigated was calculated to be the following: 1, 77% N, 23% C; 2, 31% N, 69% C; 3, 15% N, 85% C; 3A, 0% N, 100% C; 4, 97% N, 3% C; 5, 15% N, 85% C; 6, 38% N, 62% C. As a result, the two nitrogen atoms were assigned to the 1 and 4 positions, respectively.²³ The final standard residual R value for the solvated complex model was 0.0769 for observed data (2353 reflections) and 0.0985 for all data (3141 reflections). The corresponding Sheldrick R values were wR_2 of 0.2094 and 0.2269, respectively. A final difference Fourier map showed insignificant residual electron density, the largest difference peak and hole being 0.432 and –0.326 e/Å³, respectively. Final bond distances and angles were all within acceptable limits.

Synthesis of Hystatin 1 (Disodium Isoaaptamine 9-O-Phosphate) (7**).** To a dry flask equipped with a septum, magnetic stirrer, thermometer, and Ar inlet containing dry acetonitrile (20 mL) was added iso-aaptamine hydrochloride (**4**, 0.26 g, 0.97 mmol). Upon cooling to –10 °C, tetrachloromethane (0.47 mL, 4.85 mmol) was added followed by diisopropylethylamine (0.51 mL, 2.91 mmol) and a catalytic amount of (dimethylamino)pyridine. After 2 min, dropwise addition of dibenzyl phosphite (0.30 mL, 1.36 mmol) was begun while maintaining a temperature of –10 °C. The resultant yellow solution was stirred for 2 h, and 0.5 M aqueous KH₂PO₄ (30 mL) was added. The water layer was extracted with CH₂Cl₂ (4 × 50 mL), and the combined organic extract was washed with brine (100 mL). The organic layer was dried, and the solvent was removed in vacuo. Purification by column chromatography (neutral alumina, CH₂Cl₂–CH₃OH, 20:1, as eluent) gave the dibenzyl phosphate as a yellow oil (0.27 g), which was immediately dissolved in dichloromethane. Bromotrimethylsilane (0.2 mL) was added at room temperature. After stirring for 2 h, the solvent was removed and the residue was dissolved in water (20 mL). The water layer was washed with dichloromethane (3 × 10 mL), and the water was removed. The yellow solid was dissolved in methanol, a 30% solution of sodium methoxide (0.22 mL, 1.16 mmol) was added, and the mixture was stirred for 12 h. The reaction mixture was concentrated to a yellow solid that was recrystallized from acetone–water. The iso-aaptamine prodrug (**7**) was obtained as a yellow-green powder (0.15 g, 45% yield): mp 166–168 °C (dec); UV (CH₃OH) λ_{max} (log ϵ) 209 nm (4.22), 241 (4.17), 260 (4.28), 316 (3.49), 326 (3.39), 389 (3.78); IR (KBr) ν 3406 (s), 3227 (m), 1649 (s), 1604 (s), 1525 (w), 1465 (m), 1429 (w), 1342 (m), 1298 (m), 1240 (w), 1111 (s), 977 (s), 848 (m), 723 (m), 630 (m), 563 (m); ¹H NMR (D₂O and a few drops of CD₃OD) δ 3.77 (s, 3H, NMe), 3.87 (s, 3H, OMe), 5.84 (d, $J = 7.5$ Hz, 1H, H-3), 6.47 (d, $J = 7.5$ Hz, 1H, H-6), 6.62 (d, $J = 7.5$ Hz, 1H, H-5), 6.65 (s, 1H, H-7), 7.43 (d, $J = 7.5$ Hz, 1H, H-2); ¹³C NMR (D₂O) δ 45.81, 56.32, 98.05, 101.49, 112.90, 117.34, 128.96, 132.80, 134.30, 147.76, 148.32, 158.62; ³¹P NMR (162 MHz, D₂O) δ 1.58; EIMS m/z 228 (100) [$M^+ - (\text{NaO})_2\text{P(O)H}$], 213

(58), 185 (39), 170 (24), 142 (13), 114 (12), 28 (31); HRFABMS [$M - Na_2O_3P + H$] m/z 229.09392 (calcd for $C_{13}H_{13}N_2O_2$, 229.09771).

Antimicrobial Susceptibility Testing. Compounds were screened against the bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Micrococcus luteus*, *Escherichia coli*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, and *Neisseria gonorrhoeae* and the fungi *Candida albicans* and *Cryptococcus neoformans*, according to established broth microdilution susceptibility assays.^{24,25} The minimum inhibitory concentration was defined as the lowest concentration of compound that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days.

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Supporting Information Available: Illustrations of the crystal structure of isoaptamine (4) picrate-toluene complex and of the overlapping toluene conformers are given, together with tables of X-ray coordinates, bond lengths and angles, and thermal parameters. This material is available free of charge via the Internet at <http://pubs.ac-s.org>.

References and Notes

- (1) For part 379 of this series see: Pettit, G. R.; Toki, B.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **1998**, *41*, 1688–1695.
- (2) Kerr, R. G.; Kerr, S. S. *Expert Opin. Ther. Pat.* **1999**, *9*, 1207–1222.
- (3) Bongiorno, L.; Pietra, F. *Chem. Ind.* **1996**, 54–58.
- (4) (a) Faulkner, D. *J. Nat. Prod. Rep.* **2001**, *18*, 1–49. (b) Faulkner, D. *J. Nat. Prod. Rep.* **2000**, *17*, 7–55. (c) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771–1791.
- (5) Pettit, G. R.; Hogan-Pierson, F.; Herald, C. L. *Anticancer Drugs from Animals, Plants, and Microorganisms*; Wiley-Interscience: New York, 1993.
- (6) (a) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature* **1970**, *227*, 962–963. (b) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812–821. (c) Pettit, G. R. Evolutionary Biosynthesis of Anticancer Drugs. In *Anticancer Agents: Frontiers in Cancer Chemotherapy*; Ojima, I., Vite, G. D., Altmann, K.-H., Eds.; American Chemical Society: Washington, DC, 2001; pp 16–42.
- (7) (a) Pettit, G. R.; Knight, J. C.; Collins, J. C.; Herald, D. L.; Pettit, R. K.; Boyd, M. R.; Young, V. G. *J. Nat. Prod.* **2000**, *63*, 793–798. (b) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem.* **1993**, *58*, 1302–1304. (c) Pettit, G. R.; Cichacz, Z. A.; Herald, C. L.; Gao, F.; Boyd, M. R.; Schmidt, J. M.; Hamel, E.; Bai, R. *J. Chem. Soc., Chem. Commun.* **1994**, 1605–1606.

- (8) (a) Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Hoard, M. S.; Melody, N.; Pettit, R. K. *J. Nat. Prod.* **1998**, *61*, 13–16. (b) Pettit, G. R.; McNulty, J.; Herald, D. L.; Doubek, D. L.; Chapuis, J.-C.; Schmidt, J. M.; Tackett, L. P.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 180–183. (c) Pettit, G. R.; Tan, R.; Gao, F.; Williams, M. D.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J. N. A.; Tackett, L. P. *J. Org. Chem.* **1993**, *58*, 2538–2543.
- (9) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *J. Chem. Soc., Perkin Trans. 1* **1987**, 173–176.
- (10) (a) Aaptamine was first isolated from *Aaptos aaptos*. Nakamura, H.; Kobayashi, J.; Ohizumi, Y. *Tetrahedron Lett.* **1982**, *23*, 5555–5558. Subsequently, it has been isolated from certain other sponge species: (b) Berquist, P. R.; Combie, R. C.; Kernan, M. R. *Biochem. Syst. Ecol.* **1991**, *19*, 289–290. (c) Mohamed, R.; Lee, L. J.; Omar, S. *Proc. 16th Malays. Biochem. Soc. Conf.* **1991**, 37–42. (d) Park, S. K.; Kim, S. S.; Park, J. D.; Hong, J. S.; Kim, I. K. *J. Korean Chem. Soc.* **1995**, *39*, 559–563.
- (11) (a) Fedoreev, S. A.; Prokofeva, N. G.; Denisenko, V. A.; Rebachuk, N. M. *Khim.-Farm. Zh.* **1988**, *22*, 943–946. (b) Kashman, Y.; Rudi, A.; Hirsh, S.; Isaacs, S.; Green, D.; Blasberger, D.; Carmely, S. *New J. Chem.* **1990**, *14*, 729–740. (c) Shen, Y.-C.; Chein, C.-C.; Hsieh, P.-W.; Duh, C.-Y. *Taiwan Shuichan Xuehuikan* **1997**, *24*, 117–125. (d) Shen, Y.-C.; Lin, T.-T.; Sheu, J.-H.; Duh, C. Y. *J. Nat. Prod.* **1999**, *62*, 1264–1267.
- (12) Walz, A. J.; Sundberg, R. J. *J. Org. Chem.* **2000**, *65*, 8001–8010.
- (13) (a) Pettit, G. R.; Lippert, J. W., III. *Anti-Cancer Drug Des.* **2000**, *15*, 203–216. (b) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. *Tetrahedron Lett.* **1996**, *37*, 771–774.
- (14) (a) Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Chaplin, D. J.; Stratford, M.; Hamel, E.; Pettit, R. K.; Chapuis, J.-C.; Oliva, D. *Anti-Cancer Drug Des.* **1998**, *13*, 981–993. (b) Lazar, S.; Guillaumet, G. *Synth. Commun.* **1992**, *22*, 923–931.
- (15) Boyd, M. R. In *CANCER: Principles and Practices of Oncology Updates*; DeVita, V. T., Jr., Hellman, S., Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1989; Vol. 10, No. 3, pp 1–12.
- (16) Boyd, M. R. In *Current Therapy in Oncology*; Niederhuber, J. E., Ed.; B. C. Decker, Inc.: Philadelphia, 1993; pp 11–22.
- (17) Boyd, M. R.; Paull, K. D.; Rubinstein, L. R. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T., Baker, L., Eds.; Kluwer Academic Publishers: Amsterdam, 1992; pp 11–34.
- (18) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–109.
- (19) XPREP—The automatic space group determination program in the SHELXTL PC program package.
- (20) North, A. C.; Phillips, D. C.; Mathews, F. S. *Acta Crystallogr.* **1968**, *A24*, 351–359.
- (21) 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, 1992.
- (22) Sheldrick, G. M. *SHELXL93. Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1993.
- (23) Preparation of Figures S1A and S1B in the Supporting Information was done with: *SHELXTL-PC Version 5.03*, an integrated software system for the determination of crystal structures from diffraction data; Siemens Industrial Automation, Inc., Analytical Instrumentation: Madison, WI, 1994.
- (24) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7-A4. Wayne, PA: NCCLS, 1997.
- (25) National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard M27-A. Wayne, PA: NCCLS, 1997.
- (26) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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