JOC Article

# Antineoplastic Agents 491.[1] Synthetic Conversion of Aaptamine to Isoaaptamine, 9-Demethylaaptamine, and 4-Methylaaptamine[⊥]

George R. Pettit,*,† Holger Hoffmann,† Delbert L. Herald,† James McNulty,† Alison Murphy,† Kerianne C. Higgs,† Ernest Hamel,‡ Nancy E. Lewin,§ Larry V. Pearce,§ Peter M. Blumberg,§ Robin K. Pettit,† and John C. Knight†

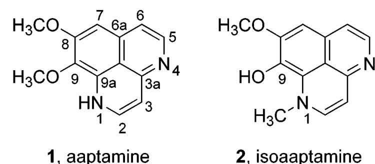
*Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 872404, Tempe, Arizona 85287-2404, Screening Technologies Branch, DTP/DCTD, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702-1201, and Molecular Mechanisms of Tumor Promotion Section, LCCTP, National Cancer Institute, Bethesda, Maryland 20892-4255*

*Received February 7, 2003*

Aaptamine (**1**) was used as starting material for synthetic transformation to isoaaptamine (**2**), 9-demethylaaptamine (**5**), and 4-methylaaptamine (**6**). A general method for the selective O-demethylation of such 1*H*-benzo[*de*][1,6]-naphthyridine (**1**) marine sponge constituents at position C-9 has been developed. Selective O-demethylation of aaptamine (**1**) and 1-methylaaptamine (**11**) with 48% hydrobromic acid led to 9-demethylaaptamine (**5**) and isoaaptamine (**2**), respectively. A selection of other aaptamine derivatives were synthesized, and their structures were unambiguously determined by X-ray methods. In addition, their cancer cell growth inhibitory properties were evaluated against the murine P388 lymphocytic cell line and a minipanel of human cancer cell lines. Evaluation as inhibitors of the PKC signal transduction pathway and against a selection of microorganisms was also undertaken. Aaptamine derivatives **3** and **5** had broad-spectrum antimicrobial activities.

## Introduction

Marine sponges are a source of many unique and potentially useful nitrogen heterocyclic constituents that include a series of 1*H*-benzo[*de*][1,6]-naphthyridines. The parent naphthyridine known as aaptamine (**1**) was first isolated by Nakamura and co-workers.[2] Subsequently, aaptamine was found to possess cancer cell growth inhibitory activity and adrenoreceptor blocking activity.[3] Another member of the series, isoaaptamine (**2**), was first reported by Fedoreev,[4] who isolated it from a sponge in the genus *Suberites*. Isoaaptamine (**2**) was later isolated from *Aaptos aaptos* by two different groups.[5] Isoaaptamine (**2**) has been reported to be a PKC inhibitor[6] and to inhibit growth of cancer cells.[5] Previously, we isolated a cancer cell line active substance from a Republic of Singapore *Hymeniacidon* sp.[7] where the $^{1}$H and $^{13}$C NMR data were close to those reported for isoaaptamine (**2**).[5] Subsequently, the structure (**2**) was unambiguously demonstrated to be isoaaptamine by an X-ray crystal structure elucidation.[7] We found isoaaptamine (**2**) to show significant activity against murine P388 lymphocytic leukemia cells ($ED_{50}$ = 0.28 $\mu$g/mL) and against a panel of six human cancer cell lines.[7] Isoaaptamine also was found to exhibit activity against a selection of clinically important microorganisms.[7] Recently, total syntheses of aaptamine (**1**)[8] and a synthesis of isoaaptamine (**2**) have been reported.[9] The first structure−activity relationship study of the aaptamines was summarized by Shen and co-workers.[10] They observed that the phenolic group at the C-9 position was important for cytotoxicity. Acylation of that position led to a decrease in activity.




Although aaptamine (**1**) and isoaaptamine differ only

* To whom correspondence should be addressed. Tel: (480) 965-3351. Fax: (480) 965-8558.

⊥ Dedicated to Professor Carl Djerassi, a great pioneer in medicinal, natural products and organic chemistry, on the occasion of his 80th birthday.

† Arizona State University.
‡ STB, NCI.
§ MMTPS, LCCTP, NCI.

(1) For contribution 490, see: Pettit, G. R.; Knight, J. C.; Herald, D. L.; Davenport, R.; Pettit, R. K.; Tucker, B. E.; Schmidt, J. M. *J. Nat. Prod.* **2002**, *65*, 1793.
(2) (a) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1982**, *23*, 5555. (b) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *J. Chem. Soc., Perkin Trans. 1* **1987**, 173.
(3) Ohizumi, Y.; Kajiwara, A.; Nakamura, H.; Kobayashi, J. *J. Pharm. Pharmacol.* **1984**, *36*, 785.
(4) Fedoreev, S. A.; Prokofeva, N. G.; Denisenko, V. A.; Rebachuk, N. M. *Khim.-Farm. Zh.* **1988**, *22*, 943.
(5) (a) Kashman, Y.; Rudi, A.; Hirsch, S.; Isaacs, S.; Green, D.; Blasberger, D.; Carmely, S. *New J. Chem.* **1990**, *14*, 729. (b) Shen, Y. C.; Chein, C. C.; Hsieh, P. W.; Duh, C. Y. *Taiwan Shuichan Xuehuikan* **1997**, *24*, 117.
(6) Patil, A. D.; Westley, J. W.; Mattern, M.; Freyer, A. J.; Hofmann, G. A. PCT Int. Appl. WO 95/0584, March 1995.
(7) Pettit, G. R.; Hoffmann, H.; McNulty, J.; Higgs, K. C.; Murphy, A.; Molloy, D. J.; Herald, D. L.; Williams, M. D.; Pettit, R. K.; Doubek, D. L.; Hooper, J. N. A.; Albright, L.; Schmidt, J. M.; Tackett, L. P. *J. Nat. Prod.*, in press.
(8) Sugino, E.; Choshi, T.; Hibino, S. *Heterocycles* **1999**, *50*, 543.
(9) Walz, A. J.; Sundberg, R. J. *J. Org. Chem.* **2000**, *65*, 8001.
(10) Shen, Y. C.; Lin, T. T.; Sheu, J. H.; Duh, C. Y. *J. Nat. Prod.* **1999**, *62*, 1264.

10.1021/jo0300486 CCC: $27.50 © 2004 American Chemical Society
Published on Web 02/28/2004

in the position of one methyl group, its shift from the C-9 position in aaptamine (**1**) to the N-1 position in isoaaptamine (**2**) leads to an increase in cytotoxicity. The skeleton of aaptamine bears four positions that could be methylated, two nitrogen atoms and two phenol groups. Therefore, 16 different methyl or demethyl derivatives of the aaptamine scaffold are possible. The aim of the present investigation was the methylation and demethylation of aaptamine (**1**) in order to explore the anticancer SAR possibilities. A parallel objective was to complete a practical methylation of aaptamine (**1**) at N-1 and selective O-demethylation at the C-9 position to provide the much less abundant isoaaptamine (**2**) in two steps. Because of our multigram isolation[7] of aaptamine (**1**) from *Hymeniacidon* sp., this marine sponge was employed as the starting material.

## Results and Discussion

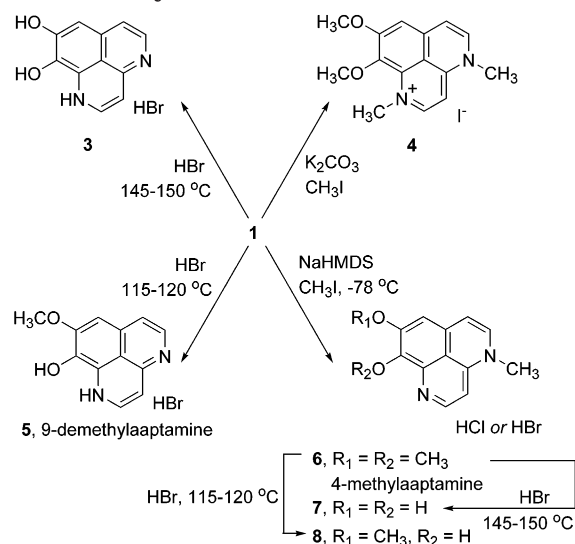
The key step in our synthesis of isoaaptamine (**2**) from aaptamine (**1**) involved selective O-demethylation at the C-9 position. Several methods are known for O-demethylation of isoquinoline derivatives. For example, Alvarez used boron tribromide in dichloromethane as part of a synthesis of batzelline B[11] and Pelletier employed hydrobromic acid for the selective O-demethylation of 6,7-dimethoxy-3,4-dihydroisoquinoline.[12] Because of the limited solubility of aaptamine (**1**) in dichloromethane, we used the latter method in a number of experiments under modified conditions. Treatment of aaptamine (**1**) with 48% hydrobromic acid at 145−150 °C yielded only diphenol **3**,[13] whereas at 115−120 °C, aaptamine (**1**) was selectively O-demethylated at the C-9 position (Scheme 2). The NMR data of 9-demethylaaptamine (**5**) were in good agreement with those reported by Nakamura et al.[2b] The structure of monophenol **5** was confirmed as the hydrobromide trihydrate via X-ray methods.

Upon successful development of the selective O-demethylation, we began N-methylation studies of aaptamine (**1**). Reaction of aaptamine (**1**) with excess methyl iodide and potassium carbonate led to formation of the permethylated quaternary ammonium salt **4**, the structure of which was again established via X-ray methods. Two new singlets appeared in the $^1$H NMR, which confirmed methylation of N-1 and N-4. Such quaternary isoquinolinium alkaloids are not unusual in nature, and they often show interesting biological activities. For example, nitidine, from the roots of *Toddalia asiatica,* showed significant anti-HIV activity and inhibited the HIV reverse transcriptase.[14]

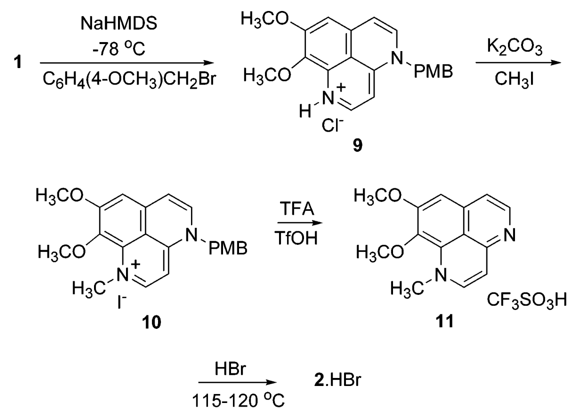
The selective methylation of aaptamine (**1**) was eventually achieved using sodium hexamethyldisilazane (NaHMDS) and methyl iodide in THF at −78 °C (Scheme 1). However, the methyl group proved to be bonded to the nitrogen atom N-4 and not to N-1, as expected an X-ray crystal structure of 4-methylaaptamine (**6**) confirmed that the methyl group was attached to nitrogen N-4. Our $^1$H and $^{13}$C NMR data of 4-methylaaptamine (**6**) were in good agreement with those reported by Coutinho et al.[15] Treatment of 4-methylaaptamine (**6**) with 48% HBr at 145−150 °C led to the formation of diphenol **7**. Selective O-demethylation of dimethyl ether **6** at position C-9 was achieved with 48% HBr at 115 °C and led to a structural isomer (**8**) of isoaaptamine (**2**). With monophenols (**2** and **8**) differing only in the position of the methyl group, the $^1$H NMR spectra were very helpful. The singlet for the *N*-methyl group of isoaaptamine (**2**) appears at 4.02 ppm and that of isomer **8** at 3.56 ppm. Although this result was interesting and potentially useful, a new strategy for the synthesis of isoaaptamine (**2**) needed to be devised (Scheme 2).

After a number of unsuccessful approaches, treatment of aaptamine (**1**) with NaHMDS and 4-methoxybenzyl bromide at −78 °C led to the PMB-protected aaptamine **9**. Reaction of the PMB-derivative with potassium carbonate and excess methyl iodide gave the desired N-1 methylated product (**10**). The position of the methyl group was unambiguously demonstrated by an X-ray structure

**SCHEME 1. Syntheses of 5 and 6**




**SCHEME 2. Synthesis of Isoaaptamine (2) from Aaptamine (1)**




(11) Alvarez, M.; Bros, M. A.; Gras, G.; Ajana, W.; Joule, J. A. *Eur. J. Org. Chem.* **1999**, *5*, 1173.
(12) Pelletier, J. C.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 616.
(13) Von Nussbaum, F.; Schumann, S.; Steglich, W. *Tetrahedron* **2001**, *57*, 2331.
(14) (a) Rashid, M.; Gustafson, K. R.; Kashman, Y.; Cardellina, J. H., II; McMahon, J. B.; Boyd, M. R. *Nat. Prod. Lett.* **1995**, *6*, 153. (b) Tan, G. T.; Pezzuto, J. M.; Kinghorn, A. D.; Hughes, S. H. *J. Nat. Prod.* **1991**, *54*, 143.
(15) Coutinho, A. F.; Chanas, B.; Souza, T. M. L.; Frugrulhetti, I. C. P. P.; Epifanio, R. de A. *Heterocycles* **2002**, *57*, 1265.
(16) Murphy, W. S.; Bertrand, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4115.

**TABLE 1. Cancer Cell Growth Inhibitory ($ED_{50}$ μg/mL) Activity of Isoaaptamine (2), 9-Demethylaaptamine (5), and Aaptamine Derivatives 3–11**

| cancer cell line | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
|---|---|---|---|---|---|---|---|---|---|---|
| leukemia P-388 | 1.6 | 0.12 | 3.9 | 0.22 | >10 | 2.2 | >10 | 2.2 | 0.23 | 3.1 |
| pancreas BXPC-3 | 4.1 | 0.33 | 7.1 | 1.6 | >10 | >10 | >10 | 4.2 | 0.74 | 7.0 |
| breast MCF-7 | 2.2 | 0.31 | 4.9 | 0.66 | >10 | 4.1 | >10 | 8.0 | 2.2 | 8.0 |
| CNS SF-268 | 2.2 | 0.80 | 0.69 | 1.4 | >10 | 5.6 | >10 | 8.2 | 0.28 | 5.9 |
| lung NCI-H460 | 2.9 | 0.52 | >10 | 2.0 | >10 | >10 | >10 | 5.1 | 1.9 | 4.6 |
| colon KM 20L2 | >10 | 0.3 | 6.1 | 1.7 | >10 | >10 | >10 | 3.9 | 0.88 | 5.4 |
| prostate DU-145 | 3.3 | 1.1 | 3.5 | 1.8 | >10 | >10 | >10 | 3.1 | 0.52 | 7.5 |

determination. In both cases, the X-ray crystal structures of aaptamine derivatives **9** and **10** confirmed that the methyl group was attached to the quaternary nitrogen N-1.

Surprisingly, cleavage of the PMB protecting group proved to be difficult. No reaction was observed using TFA at 70 °C. With ceric ammonium nitrate or 48% hydrobromic acid, only decomposition was observed. Finally, treatment of PMB-derivative **10** with a mixture of TFA and trifluoromethanesulfonic acid[16] led to removal of the PMB group. The quartet in the $^{13}C$ NMR at 121.8 ppm and the singlet in the $^{19}F$ NMR at 44.1 ppm confirmed the formation of 1-methylaaptamine (**11**) as a trifluoromethanesulfonic acid salt. Trimethyl derivative **11** was also prepared by Hibino[17a] and Sundberg.[9] The selective O-demethylation of dimethyl ester **11** with 48% HBr at 115 °C gave the natural product isoaaptamine (**2**) as the hydrobromide salt. Both $^{1}H$ and $^{13}C$ NMR spectral comparison of synthetic isoaaptamine (**2**) with a sample of natural origin confirmed that they were identical.

**Biology.** The cancer cell growth inhibitory properties were examined using the murine P388 lymphocytic leukemia cell line and a selection of human cancer cell lines. The results are summarized in Table 1. In general, O-demethylation led to an increase in inhibitory activity. For example, 9-demethylaaptamine (**5**) and diphenol **3** were more inhibitory than aaptamine (**1**).[7] Isoaaptamine (**2**) exhibited activity comparable to that of methylaaptamine **11**. Inhibitory activity was markedly reduced or eliminated by methylation of aaptamine (**1**). Methylation at N-4 led to inactive derivatives (**6** and **8**). (However, during the preparation of the final draft of our manuscript, Epifanio[15] reported the isolation of amine **6** from the marine sponge *Aaptos* sp. collected in Abrolhos, Bahia, Brazil. Importantly, 4-methylaaptamine (**6**) showed potent antiviral activity against herpes simplex virus type 1 (HSV-1) and low toxicity to Vero cells.) Interestingly, the quaternary ammonium salts **4** and **10** exhibited significant inhibitory activity against the murine P388 lymphocytic leukemia and the human cancer cell lines.

Isoaaptamine (**2**) and derivatives **3**–**11** were evaluated as ligands for PKC, on the basis of the initial report of activity for isoaaptamine.[6] Activity was measured at 30 μM compound using inhibition of [$^{3}H$-20]phorbol 12,13-dibutyrate binding to PKC α as described previously.[17b] Assays were carried out with triplicate measurements in either single or triplicate experiments, depending on the compound. Only 13% inhibition was observed for **2** and less than 10% inhibition for the other derivatives. We conclude that these compounds do not show appreciable affinity for PKC.

The series of compounds **3**–**11** described here were examined for possible effects on tubulin polymerization, under conditions in which the colchicine site agent combretastatin A-4 yielded an $IC_{50}$ value of about 2 μM for inhibition of extent of assembly. Only minimal inhibitory activity was observed. Although compounds **3** and **8** did inhibit the rate of polymerization by over 50% at 40 μM (the highest concentration examined), an $IC_{50}$ value for inhibition of assembly extent was obtained only for compound **5**. The $IC_{50}$ for compound **5** was 37 ± 4 (SD) μM, about 18-fold higher than the value obtained for combretastatin A-4. Thus, it seems unlikely that the cytotoxic mechanism for these aaptamine analogues derives from an interaction with tubulin.

9-Demethylaaptamine (**5**) is known to have activity against Gram-positive and Gram-negative bacteria.[2b] We demonstrated here that phenol **5** also has antifungal activity (Table 2). The new aaptamine derivative **3** had antibacterial and antifungal activities (Table 2). The antimicrobial activity of phenols **3** and **5** in the presence of human serum was also investigated. For both compounds, minimum inhibitory concentrations were identical or one 2-fold dilution lower than those obtained in the absence of serum.

In conclusion, convenient syntheses of isoaaptamine (**2**), 9-demethylaaptamine (**5**), and a selection of methyl derivatives have been developed. With the procedures described herein, we were able to synthesize eight of the 16 possible O and N methyl-derivatives of aaptamine. A selection of these naphthyridines were found to exhibit significant cancer cell growth and antimicrobial inhibitory activities. They did not function, however, as ligands for PKC, despite earlier reports.[6]

## Experimental Section

**General Experimental Procedures.** Principal reagents were obtained from Sigma-Aldrich Co., and solvents were distilled prior to use. Aaptamine (**1**) was isolated from *Hymeniacidon* sp. according to the procedure described previously.[7] Column chromatography was performed using either flash silica gel from EM Science (230–400 mesh ASTM) or gravity silica (70–230 mesh ASTM), aluminum oxide from Sigma-Aldrich Co. (activated, neutral, Bockmann I, ~150 mesh, 58 D), and Sephadex LH-20 from Pharmacia Fine Chemical AB (25–100 μm). Thin-layer chromatography was performed using aluminum oxide plastic sheets (E. Merck). All compounds were visible under UV light (254 nm).

Melting points were recorded employing an Electrothermal 9100 apparatus and are uncorrected. The $^{1}H$, $^{13}C$, and $^{19}F$ NMR spectra were obtained using Varian VXR-500 or VXR-400

(17) (a) Hibino, S.; Sugino, E.; Choshi, T.; Sato, K. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2429. (b) Kazanietz, M. G.; Areces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. *Mol. Pharmacol.* **1993**, *44*, 298.

**TABLE 2. Antimicrobial Activities of Aaptamine Derivatives**

| microorganism | range of minimum inhibitory concentration (ug/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| *Cryptococcus neoformans* | 32 | *a* | 32 | *a* | *a* | *a* | *a* | *a* | *a* |
| *Candida albicans* | 64 | *a* | 64 | *a* | *a* | *a* | *a* | *a* | *a* |
| *Staphylococcus aureus* | 4−8 | *a* | 16 | *a* | *a* | *a* | *a* | *a* | *a* |
| *Streptococcus pneumoniae* | 8 | *a* | 2−4 | *a* | *a* | *a* | 64 | *a* | 64 |
| *Enterococcus faecalis* | 8−16 | *a* | 8−32 | *a* | *a* | *a* | *a* | *a* | *a* |
| *Micrococcus luteus* | 4 | 16−32 | 8 | *a* | *a* | *a* | 64 | 4−8 | *a* |
| *Escherichia coli* | 16−64 | *a* | 32−64 | *a* | *a* | *a* | *a* | *a* | *a* |
| *Enterobacter cloacae* | *a* | *a* | *a* | *a* | *a* | *a* | *a* | *a* | *a* |
| *Stenotrophomonas maltophilia* | 32 | *a* | *a* | *a* | *a* | *a* | *a* | *a* | *a* |
| *Neisseria gonorrhoeae* | <0.5 | *a* | <0.5 | *a* | 64 | 4 | 64 | 64 | *a* |

[a] No inhibition at 64 ug/mL.

instruments. Mass spectral data were recorded using a Varian MAT 312 instrument (EIMS), and IR spectra were determined with a Mattson Instruments 2020 Galaxy Series FTIR instrument. The X-ray crystal structure data collections were performed on an Enraf-Nonius CAD4 diffractometer or a Bruker SMART 6000 diffractometer. Descriptions of the routine X-ray structure determinations for compounds **4**, **5**, **6**, **9**, and **10** are presented in Supporting Information. High-resolution mass spectra were obtained on a JEOL LCMate magnetic sector instrument in the APCI mode with poly(ethylene glycol) as reference or by FAB with a glycerol matrix.

**8,9-Dihydroxy-1*H*-benzo[*de*][1,6]naphthyridine Hydrobromide (3).** Aaptamine (**1**) hydrochloride (0.50 g, 1.89 mmol) was dissolved in 48% HBr (5 mL), and the solution was stirred at 145−150 °C (preheated oil bath). After 4 h the mixture was cooled to room temperature. The solution was filtered, and the solid was washed with ethyl acetate to give diphenol **3** as a green powder (0.41 g, 77%). Further purification was achieved by column chromatography on Sephadex LH-20 with $CH_3OH$ as eluent: mp 245−248 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 205 (3.93), 245 (4.08), 269 (3.96), 306 (3.31), 360 (3.33), 4.08 (3.27) nm; IR $(KBr)_{max}$ 1655, 1626, 1556, 1444 $cm^{-1}$; $^1H$ NMR (500 MHZ, $CD_3OD$) $\delta$ 6.16 (d, $J$ = 7.5 Hz, 1H, H-3), 6.61 (d, $J$ = 7.3 Hz, 1H, H-6), 6.73 (s, 1H, H-7), 6.98 (d, $J$ = 7.3 Hz, 1H, H-5), 7.63 (d, $J$ = 7.5 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $CD_3OD$) $\delta$ 98.1, 105.3, 113.9, 117.7, 128.1, 129.8, 130.9 (2C), 142.2, 151.6, 152.4; EIMS $m/z$ 200 (100) [$M^+$], 171 (42), 154 (15), 82 (25), 80 (26), 28 (64); HRMS [$APCI^+$] $m/z$ $[M - Br]^+$ 201.0670 (calcd for $C_{11}H_9N_2O_2$, 201.0664).

**1,4-Dimethyl-8,9-dimethoxy-4*H*-benzo-[*de*][1,6]-naphthyridin-1-ium Iodide (4).** Aaptamine (**1**) hydrochloride (0.50 g, 1.89 mmol) was dissolved in anhydrous dimethylformamide (50 mL). Potassium carbonate (1.31 g, 9.45 mmol) and methyl iodide (0.56 mL, 9.45 mmol) were added at room temperature. After stirring for 12 h at the same temperature, the solution was filtered, and the solvent was removed in vacuo to leave a brown oil. The residue was purified by column chromatography (silica gel, $CH_2Cl_2-CH_3OH$ 6:1) to give the product as a bright yellow solid (0.60 g, 82%). Yellow needles were obtained by crystallization from $CH_2Cl_2-CH_3OH$: mp 215−217 °C (dec); $R_f$ 0.53 ($CH_2CL_2-CH_3OH$ 10:1); UV ($CH_3OH$) $\lambda_{max}$ log $\epsilon$) 208 (4.39), 220 (4.45), 241 (4.38), 261 (4.39), 271 (4.38), 279 (4.35), 397 (3.84) nm; IR $(KBr)_{max}$ 1647, 1570, 1350, 1305, 1084 $cm^{-1}$; $^1H$ NMR (500 MHZ, $CD_3OD$) $\delta$ 3.63 (s, 3H, $CH_3$), 3.77 (s, 3H, $CH_3$), 3.98 (s, 3H, $CH_3$), 4.04 (s, 3H, $CH_3$), 6.49 (d, $J$ = 7.8 Hz, 1H, H-3), 6.91 (d, $J$ = 7.3 Hz, 1H, H-6), 7.25 (s, 1H, H-7), 7.54 (d, $J$ = 7.3 Hz, 1H, H-5), 8.09 (d, $J$ = 7.8 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $CD_3OD$) $\delta$ 41.1, 45.7, 56.6, 62.2, 96.9, 102.4, 113.2, 117.8, 132.3, 133.8, 133.9, 135.3, 148.9, 149.4, 158.1; EIMS $m/z$ 257 (1) [$M^+ - I$], 242 (60), 227 (83), 199 (40), 184 (37), 142 (100), 127 (50), 28 (46); HRFABMS $m/z$ $[M - I]^+$ 257.1275 (calcd for $C_{15}H_{17}N_2O_2$, 257.1290).

**9-Demethylaaptamine Hydrobromide (5).** Aaptamine (**1**) hydrochloride (0.21 g, 0.79 mmol) was dissolved in 48% HBr (3 mL). After stirring for 45 min at 115−120 °C (preheated oil bath), the solvent was removed in vacuo to leave a brown solid. Addition of $CH_2Cl_2-CH_3OH$ (10:1) led to the precipitation of a yellow solid. Collection by filtration gave 9-demethylaaptamine (**5**) hydrobromide as a yellow-green amorphous powder (0.15 g, 64%). Further purification was achieved by column chromatography (silica gel, $CH_2Cl_2-CH_3OH$ 6:1): mp 260−264 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 209 (4.16), 244 (4.26), 268 (4.07), 276 (4.02), 316 (3.37), 366 (3.52), 410 (3.47) nm; IR $(KBr)_{max}$ 3149, 3074, 3020, 1656, 1604, 1552, 1323, 1238 $cm^{-1}$; $^1H$ NMR (500 MHZ, $CD_3OD$) $\delta$ 4.01 (s, 3H, $OCH_3$), 6.16 (d, $J$ = 6.5 Hz, 1H, H-3), 6.71 (d, $J$ − 7.3 Hz, 1H, H-6), 6.92 (s, 1H, H-7), 7.02 (d, $J$ = 7.3 Hz, 1H, H-5), 7.63 (d, $J$ = 6.5 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $CD_3OD$) $\delta$ 57.1, 98.2, 101.6, 114.6, 118.2, 128.2, 129.7, 129,8, 131.7, 142.6, 151.7, 153.5; EIMS $m/z$ 214 (100) [$M^+$], 200 (60), 199 (42), 171 (70), 142 (25), 96 (30), 94 (28), 82 (55), 80 (60); HRFABMS $m/z$ $[M - Br]^+$ 215.0803 (calcd for $C_{12}H_{11}N_2O_2$, 215.08206).

**4-Methylaaptamine (6).** Under an argon atmosphere, aaptamine (**1**) hydrochloride (1.00 g, 3.78 mmol) was suspended in anhydrous tetrahydrofuran (20 mL) and NaHMDS (7.94 mL, 7.94 mmol, 1.0 M in THF) was added at room temperature. The mixture was stirred for 15 min at the same temperature and then cooled to −78 °C. Methyl iodide (0.28 mL, 4.54 mmol) was added via syringe, and the solution was stirred for 3 h at −78 °C. Next, the mixture was allowed to warm to room temperature and was stirred for 1 h. The reaction was terminated with a 1.0 M HCl solution (10 mL), and the solvent was removed in vacuo. The residue was separated by column chromatography (silica gel, $CH_2Cl_2-CH_3OH$, 6:1). An analytical sample was obtained by crystallization from ethyl acetate−methanol with slow evaporation of the solvent (0.81 g, 77%): mp 225−227 °C (dec); $R_f$ 0.78 ($CH_2Cl_2-CH_3OH$, 10:1); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 218 (3.91), 237 (3.82), 257 (3.70), 268 (3.65), 277 (3.61), 313 (3.00), 359 (3.11), 394 (3.10) nm: IR $(KBr)_{max}$ 1653, 1601, 1465, 1321, 1095 $cm^{-1}$; $^1H$ NMR (400 MHZ, $D_6$-DMSO) $\delta$ 3.63 (s, 3H, $NCH_3$), 3.82 (s, 3H, $OCH_3$), 3.98 (s, 3H, $OCH_3$), 6.44 (d, $J$ = 7.2 Hz, 1H, H-3), 6.92 (d, $J$ = 7.4 Hz, 1H, H-6), 7.16 (s, 1H, H-7), 7.49 (d, $J$ = 7.4 Hz, 1H, H-5), 8.00 (d, $J$ = 7.2 Hz, 1H, H-2), 12.46 (br s, 1H, NH); $^{13}C$ NMR (101 MHZ, $D_6$-DMSO) $\delta$ 40.7, 56.5, 60.4, 96.7, 101.2, 113.0, 116.6, 131.3, 131.6, 133.1, 135.4, 142.7, 149.9, 156.3; EIMS $m/z$ 242 (84) [$M^+$], 227 (100) [$M^+ -$ Me], 213 (36), 184 (42), 183 (43), 128 (48), 127 (40), 28 (65); HRFABMS $m/z$ $[M - Cl]^+$ 243.11373 (calcd for $C_{14}H_{15}N_2O_2$, 243.11335).

**4-Methyl-8,9-dihydroxy-4*H*-benzo[*de*][1,6]naphthyridine (7) Hydrobromide.** The preceding 4-methylaaptamine (**6**, 0.50 g, 1.79 mmol) was dissolved in 48% HBr (5.0 mL), and the brown solution was stirred for 6 h at 150 °C (preheated oil bath). The solution was filtered, and the solvent was removed in vacuo to leave a brown solid which was purified by column chromatography (Sephadex LH-20, $CH_3OH$). Diphenol **7** was obtained as a yellow amorphous powder (0.40 g, 75%): mp 250−253 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 208 (4.10), 242 (4.28), 273 (4.11), 360 (3.52), 419 (3.37) nm; IR

$(KBr)_{max}$ 1660 (s), 1608 (s), 1240 (s) $cm^{-1}$; $^1H$ NMR (400 MHZ, $D_6$-DMSO) $\delta$ 3.53 (s, 3H, $NCH_3$), 6.26 (d, $J$ = 7.2 Hz, 1H, H-3), 6.76 (d, $J$ = 7.4 Hz, 1H, H-6), 6.85 (s, 1H, H-7), 7.25 (d, $J$ = 7.4 Hz, 1H, H-5), 7.84 (t, $J$ = 6.6 Hz, 1H, H-2), 9.88 (br s, 1H, OH), 11.02 (br s, 1H, OH), 12.14 (d, $J$ = 6.0 Hz, 1H, NH); $^{13}C$ NMR (101 MHZ, $D_6$-DMSO) $\delta$ 40.3, 95.7, 104.1, 112.6, 116.2, 126.7, 128.9, 129.2, 133.3, 142.1, 149.8, 150.2; EIMS $m/z$ 214 (100) [$M^+$ − HBr], 199 (65), 185 (13), 171 (58), 142 (13), 82 (23), 80 (25), 28 (63); HRFABMS $m/z$ [M − Br]$^+$ 215.0818 (calcd for $C_{12}H_{11}N_2O_2$, 215.08206).

**4-Methyl-8-methoxy-9-hydroxy-4*H*-benzo[*de*][1,6]-naphthyridine (8) Hydrobromide**. A solution of 4-methylaaptamine (**6**) hydrochloride (0.20 g, 0.71 mmol) in 48% HBr (3 mL) was stirred at 115−120 °C (preheated oil bath) for 1 h (none of the starting material could be detected by TLC). The solvent was removed in vacuo to leave a brown oil, which was separated by column chromatography (silica gel, $CH_2Cl_2$−$CH_3OH$, 6:1). The product was obtained as a brown solid (0.18 g, 82%), which was washed with $CH_2Cl_2$−$CH_3OH$ (20:1). Collection of the solid by filtration gave phenol **8** as a green amorphous powder: mp 252−255 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 209 (3.87), 244 (4.02), 268 (3.85), 316 (3.30), 366 (3.28), 410 (3.24) nm; IR $(KBr)_{max}$ 3396, 1658, 1618, 1242, 1089 $cm^{-1}$; $^1H$ NMR (400 MHZ, $D_6$-DMSO) $\delta$ 3.56 (s, 3H, $NCH_3$), 3.97 (s, 3H, $OCH_3$), 6.29 (d, $J$ 6.4 Hz, 1H, H-3), 6.83 (d, $J$ = 7.0 Hz, 1H, H-6), 7.13 (s, 1H, H-7), 7.34 (d, $J$ = 7.0 Hz, 1H, H-5), 7.87 (br t, 1H, H-2), 10.15 (s, 1H, OH), 12.19 (s, 1H, NH); $^{13}C$ NMR (101 MHZ, $CD_3OD$) $\delta$ 41.3, 57.1, 96.5, 101.7, 115.0, 118.4, 128.4, 128.9, 131.7, 134.1, 143.1, 151.7, 152.7; EIMS $m/z$ 228 (90) [$M^+$], 210 (51) [$M^+$ − $H_2O$], 185 (51), 170 (28), 96 (63), 94 (67), 28 (100); HRMS [$APCI^+$] $m/z$ [M − Br]$^+$ 229.0985 (calcd for $C_{13}H_{13}N_2O_2$, 229.0977).

**4-(4-Methoxybenzyl)-8,9-dimethoxy-4*H*-benzo[*de*][1,6]-naphthyridine (9) Hydrochloride.** Under an argon atmosphere, aaptamine (**1**) hydrochloride (1.00 g, 3.78 mmol) was suspended in anhydrous tetrahydrofuran (20 mL), and sodium hexamethyldisilazane (7.94 mL, 7.94 mmol, 1.0 M in THF) was added at room temperature. The mixture was stirred for 15 min at the same temperature and was then cooled to −78 °C. Next, 4-methoxybenzyl bromide (0.91 g, 4.54 mmol) in THF (5 mL) was added (dropwise), and the solution was stirred for 3 h at −78 °C. Afterward, the reaction mixture was allowed to warm to room temperature and was stirred for 1 h. The reaction was stopped with a 1.0 M HCl solution (10 mL), and the solvent was removed in vacuo. The residue was separated by column chromatography (silica gel, $CH_2Cl_2$−MeOH, 6:1). Crystallization from ethyl acetate−methanol with slow evaporation of the solvent gave the PMB derivative of **9** as a yellow amorphous powder (0.95 g, 65%): mp 235−237 °C (dec); $R_f$ 0.81 ($CH_2Cl_2$−$CH_3OH$, 10:1); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 205 (3.97), 241 (4.04), 261 (4.13), 269 (4.14), 359 (3.26), 395 (3.26) nm; IR (KBr) 1595, 1250 $cm^{-1}$; $^1H$ NMR (500 MHZ, $CD_3OD$) $\delta$ 3.76 (s, 3H, $OCH_3$), 3.93 (s, 3H, $OCH_3$), 4.05 (s, 3H, $OCH_3$), 5.21 (s, 2H, $NCH_2$), 6.45 (d, $J$ = 7.3 Hz, 1H, H-3), 6.94 (d, $J$ = 9.0 Hz, 2H, $C_{ar}$), 6.97 (d, $J$ = 7.5 Hz, 1H, H-6), 7.14 (s, 1H, H-7), 7.25 (d, $J$ = 8.5 Hz, 2H, $C_{ar}$), 7.45 (d, $J$ = 7.5 Hz, 1H, H-5), 7.83 (d, $J$ = 7.3 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $CD_3OD$) $\delta$ 55.8, 57.0, 57.1, 61.3, 98.2, 102.9, 115.2, 115.7, 118.9, 127.0, 129.7, 132.9, 133.9, 135.0, 135.6, 143.2, 151.7, 158.4, 161.4; EIMS $m/z$ 348 (27) [M − Cl], 167 (60), 121 (100), 28 (50); HRMS [$APCI^+$] $m/z$ [M − Cl]$^+$ 349.15364 (calcd for $C_{21}H_{21}N_2O_3$, 349.15522).

**1-Methyl-4-(4-methoxybenzyl)-8,9-dimethoxy-4*H*-benzo[*de*][1,6]-naphthyridin-1-ium Iodide (10).** To a suspension of 4-(4-methoxybenzyl)-aaptamine (**9**) hydrochloride (1.00 g, 2.60 mmol) in anhydrous dimethylformamide (50 mL) were added potassium carbonate (1.80 g, 13.00 mmol) and methyl iodide (0.81 mL, 13.00 mmol) at room temperature. After stirring for 12 h the solution was filtered, and the solvent was removed in vacuo. The oily residue was separated by column chromatography (silica gel, $CH_2Cl_2$−$CH_3OH$, 6:1) to give quaternary iodide **10** (1.15 g, 94%): mp 189−191 °C (dec); $R_f$ 0.72 ($CH_2Cl_2$−$CH_3OH$, 10:1); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 221 (4.37), 262 (4.19), 271 (4.18), 307 (3.46), 413 (3.64) nm; IR $(KBr)_{max}$ 1647, 1612, 1568, 1526, 1464, 1348, 1308, 1260, 1097, 1055 $cm^{-1}$; $^1H$ NMR (400 MHZ, $CD_3OD$) $\delta$ (s, 3H, $CH_3$), 3.86 (s, 3H, $CH_3$), 4.06 (s, 3H, $CH_3$), 4.09 (s, 3H, $CH_3$), 5.23 (2H, $NCH_2$), 6.42 (d, $J$ = 9.5 Hz, 1H, H-3), 6.93 (d, $J$ = 11 Hz, 2H, $C_{ar}$), 7.02 (d, $J$ = 9.3 Hz, 1H, H-6), 7.23 (d, $J$ = 11 Hz, 2H, $C_{ar}$), 7.24 (s, 1H, H-7), 7.51 (d, $J$ = 9.3 Hz, 1H, H-5), 7.79 (d, $J$ = 9.5 Hz, 1H, H-2); $^{13}C$ NMR (101 MHZ, $CD_3OD$) $\delta$ 47.0, 55.8, 57.2, 57.3, 63.0, 98.5, 104.1, 115.4, 115.7, 120.2, 126.9, 129.7, 134.1, 135.5, 136.4, 150.4, 150.8, 160.5, 161.4; EIMS $m/z$ 363 (4) [M − I], 227 (44), 142 (78), 121 (100), 28 (46); HRFABMS $m/z$ [M − I]$^+$ 363.17295 (calcd for $C_{22}H_{23}N_2O_3$, 363.17087).

**1-Methyl-8,9-dimethoxy-1*H*-benzo[*de*][1,6]naphthyridine Trifluoromethanesulfonate (11).** To a solution of the quaternary ammonium salt **10** (1.10 g, 2.48 mmol) in trifluoroacetic acid (10 mL) at room temperature was added trifluoromethanesulfonic acid (1 mL), and the mixture was stirred at 75 °C. The color of the mixture became deep red. After 1 h the trifluoromethane sulfonic acid was removed (in vacuo) to leave a red oil, which was dissolved in dichloromethane (50 mL). The organic layer was washed successively with 10% $NaHCO_3$ (50 mL) and brine (50 mL) and dried (anhydrous $MgSO_4$). The solvent was removed (in vacuo) to leave a brown oil, which was separated by column chromatography (silica gel, $CH_2Cl_2$−$CH_3OH$, 6:1). The product was obtained as a fine yellow powder (0.68 g, 70%). Fine yellow needles were obtained by crystallization from EtOAc−$CH_3OH$: mp 253−255 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 206 (4.16), 2.59 (4.31), 315 (3.59), 390 (3.76) nm; IR $(KBr)_{max}$ 1288, 1246, 1161, 1030, 636 $cm^{-1}$; $^1H$ NMR (500 MHZ, $CD_3OD$) $\delta$ 3.84 (s, 3H, $CH_3$), 4.03 (s, 3H, $CH_3$), 4.04 (s, 3H, $CH_3$), 6.26 (d, $J$ = 7.8 Hz, 1H, H-3), 6.86 (d, $J$ = 7.5 Hz, 1H, H-6), 7.08 (s, 1H, H-7), 7.23 (d, $J$ = 7.5 Hz, 1H, H-5), 7.68 (d, $J$ = 7.8 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $CD_3OD$) $\delta$ 46.55, 57.12, 62.93, 99.35, 103.51, 114.71, 119.14, 121.81 (q, $J$ = 318.3 Hz, $CF_3SO_3H$), 130.01, 135.36, 135.83, 136.01, 149.92, 150.62, 160.88; $^{19}F$ NMR (470 MHZ, $CD_3OD$) $\delta$ 44.06 (s, $CF_3SO_3H$); EIMS $m/z$ 242 (67) [$M^+$], 227 (100) [$M^+$ − Me], 184 (55), 28 (49); HRMS [$APCI^+$] $m/z$ [M − $CF_3SO_3$]$^+$ 243.1127 (calcd for $C_{14}H_{15}N_2O_2$, 243.1134).

**Isoaaptamine Hydrobromide (2).** A solution of 1-methylaaptamine trifluoromethanesulfonate (**11**) (0.62 g, 1.58 mmol) in 48% HBr (5 mL) was stirred at 115−120 °C (preheated oil bath). After 1 h, the solvent was removed, and the brown oily residue was separated by column chromatography (silica gel, $CH_2Cl_2$−$CH_3OH$, 6:1). Isoaaptamine (**2**) hydrobromide was obtained as a yellow amorphous powder (0.40 g, 81%): mp 225−227 °C (dec); UV ($CH_3OH$) $\lambda_{max}$ (log $\epsilon$) 209 (4.19), 246 (4.23), 270 (4.13), 321 (3.61), 414 (3.64) nm; IR $(KBr)_{max}$ 1647, 1595, 1300, 1244, 1195 $cm^{-1}$; $^1H$ NMR (50 MHZ, $D_6$-DMSO) $\delta$ 3.95 (s, 3H, $OCH_3$), 4.02 (s, 3H, $NCH_3$), 6.20 (d, $J$ = 7.5 Hz, 1H, H-3), 6.77 (d, $J$ = 7.3 Hz, 1H, H-6), 7.12 (s, 1H, H-7), 7.22 (d, $J$ = 7.3 Hz, 1H, H-5), 7.73 (d, $J$ = 7.5 Hz, 1H, H-2); $^{13}C$ NMR (126 MHZ, $D_6$-DMSO) $\delta$ 46.0, 56.6, 97.3, 101.5, 113.1, 118.0, 127.8, 129.2, 129.3, 132.2, 149.0, 149.2, 153.5; EIMS $m/z$ 228 (100) [$M^+$], 213 (54) [$M^+$ − Me], 185 (35), 170 (21), 28 (46).

**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.[18,19] The mini-

(18) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard M7-A4. NCCLS: Wayne, PA, 1997.

(19) National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A. NCCLS: Wayne, PA, 1997.

mum 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.

**Acknowledgment.** We are pleased to thank the following for very necessary financial support: Outstanding Investigator Grant CA44344-05-12 and grant RO1 CA90441-01 awarded by the Division of Cancer Treatment and Diagnosis, National Cancer Institute DHHS; the Arizona Disease Control Research Commission; the Fannie E. Rippel Foundation; the Robert B. Dalton Endowment Fund; the Caitlin Robb Foundation, Gary L. and Diane Tooker; Polly J. Trautman; Billie Jane Baguley; Lotte Flugel; Dr. John C. Budzinski; the Eagles Art Ehrmann Cancer Fund; and the Ladies Auxiliary to the Veterans of Foreign Wars. For other helpful assistance, we thank Drs. Jean-Charles Chapuis, Fiona Hogan, and Jean Schmidt, Lee Williams, and Bridget Fakoury.

**Supporting Information Available:** Experimental details of X-ray Crystal Structure Determination of **4**, **5**, **6**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0300486