Nucleosides and Nucleotides. 96. Synthesis and Antitumor Activity of 5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) and Its Derivatives[1]

Noriaki Minakawa,[†] Takayuki Takeda,[†] Takuma Sasaki,[‡] Akira Matsuda,*[,†] and Tohru Ueda[†]

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-Ku, Sapporo 060, and Cancer Research Institute, Kanazawa University, Takara-machi, Kanazawa 920, Japan. *Received June 7, 1990*

The palladium-catalyzed cross-coupling reaction of 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**8**) with various terminal alkynes in the presence of bis(benzonitrile)palladium dichloride in acetonitrile containing triethylamine gave the desired 5-alkynyl derivatives **9** in high yields. However, when (trimethylsilyl)acetylene was used, the only isolable product was the undesired dimer, 1,2-bis(4-carbamoyl-1-β-D-ribofuranosylimidazol-5-yl)acetylene derivative **10a**. To circumvent such dimer formation, the reaction was done with use of trimethyl-[(tributylstannyl)ethynyl]silane in the absence of triethylamine to afford the desired 5-(2-trimethylsilyl)ethynyl derivative **9a** in good yield. Furthermore, the similar cross-coupling reaction of 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**12**) with (trimethylsilyl)acetylene also afforded the desired nucleoside **13a**. Deprotection of these compounds furnished 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (**6b–k**) and -carbonitriles (**14b–f**). Among these, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6b**, EICAR) is the most potent inhibitor of growth of the various tumor cells in culture including human solid tumor cells. Preliminary results of in vivo antitumor activity against murine leukemias L1210 and P388 are also described.

Inosine 5′-monophosphate (IMP) dehydrogenase catalyzes the oxidative conversion of IMP to xanthosine 5′-monophosphate (XMP) and is one of the key rate-controlling enzymes of de novo guanine nucleotide biosynthesis in mammalian systems. Weber has recently reported that the IMP dehydrogenase activity in hepatoma 3683-F is about 10–14 times higher than that found in normal rat liver.[2] Therefore, IMP dehydrogenase has been suggested as one of the target enzymes for cancer chemotherapy.[3,4] This enzyme has also been considered as the target enzyme for antiviral[5,6] as well as antiparasitic chemotherapy.[7,8]

Nucleoside analogues with a five-membered heterocycle as the base moiety, such as tiazofurin (**2**), ribavirin (**3**), and bredinin (**4**) (Chart I), are cytotoxic to certain cancer cells in vitro and in vivo,[9] potent inhibitors of proliferation of certain RNA viruses,[10] and immunosuppressors,[11] respectively. These nucleosides are structurally similar to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**1**, AICAR), the 5′-monophosphate of which is a key intermediate in purine biosynthesis, and all of these nucleoside 5′-monophosphates are potent inhibitors of IMP dehydrogenase.[12-14] Tiazofurin (**2**) is uniquely metabolized in cells to thiazole adenine dinucleotide (TAD), which is a much more potent inhibitor of IMP dehydrogenase, since it is structurally quite similar to nicotinamide adenine dinucleotide, reduced form (NADH), which is required for the enzyme reaction to convert IMP to XMP.[12] On the other hand, showdomycin (**5**), a naturally occurring maleimide C-nucleoside, acts as an alkylating agent for sulfhydryl groups, but is not a substrate for nucleoside kinases.[15]

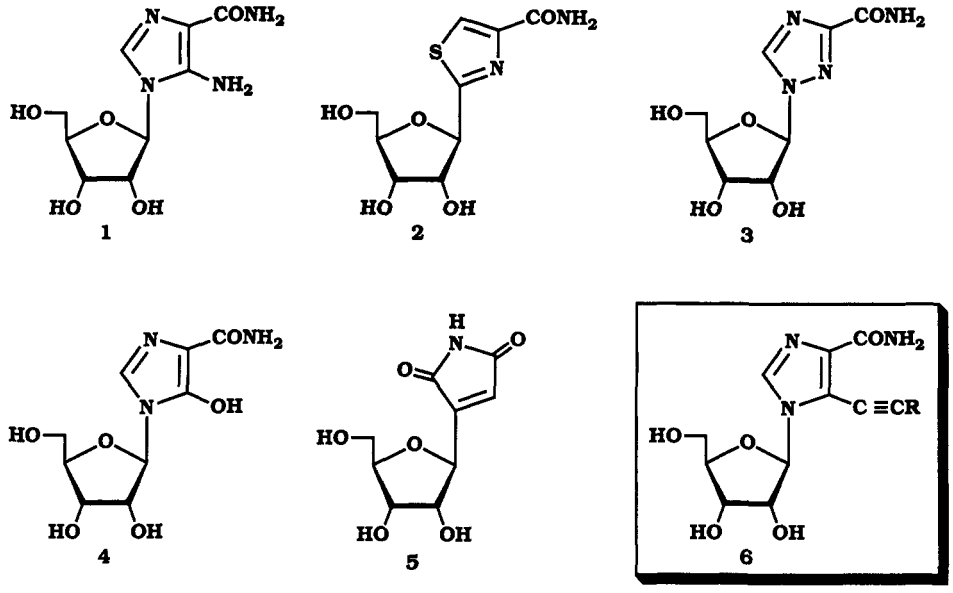
From these considerations, one approach to the design of nucleoside analogues that have properties both as antimetabolites and alkylators has been the construction of analogues that (a) are structurally similar to AICAR, (b) are converted into the corresponding 5′-monophosphates by nucleoside kinases, which should inhibit IMP dehydrogenase, and (c) have a chemically reactive functional group that acts as an alkylating reagent to cause irreversible inhibition of the enzyme action. As an example of compounds that fulfull, or partly fulfill, these requirements, we designed 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides, which have a chemically reactive alkynyl group at the 5-position of AICAR instead of the amino group. Quite recently, Hedstrom and Wong proposed a mechanism for IMP dehydrogenase that would involve nucleophilic addition of an enzyme cysteine thiol or hydroxide anion to the 2-position of IMP, followed by hydride transfer to NAD.[8] If the former is the case, it is expected that the alkynyl group at 5-position of the nucleosides designed could react with a sulfhydryl group of the enzyme. As examples, several alkynylated nucleosides have been synthesized and found to have interesting biological activites. 5-Ethynyl-2′-deoxyuridine 5′-monophosphate is a potent inhibitor of thymidylate synthetase, the sulfhydryl group of which at the active site attacks at the 6-position of the nucleotide, transiently forming a highly reactive α-keto allene system.[16] Synthesis and

* Author to whom correspondence should be addressed.
† Hokkaido University.
‡ Kanazawa University.

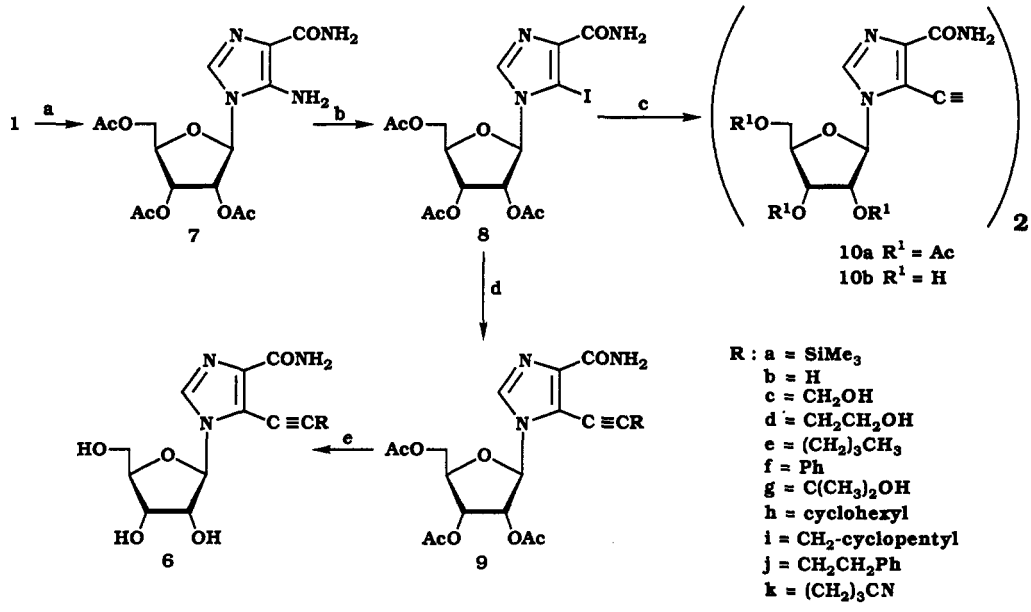
(1) Part 95; Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. *J. Med. Chem.* In press.
(2) Weber, G. *J. Med. Chem.* **1977**, *20*, 486, 541.
(3) (a) Jackson, R. C.; Morris, H. P.; Weber, G. *Biochem. J.* **1977**, *166*, 1. (b) Weber, G. *Cancer Res.* **1983**, *43*, 3466.
(4) Robins, R. K. *Nucleosides Nucleotides* **1982**, *1*, 35.
(5) Streeter, D. G.; Witkowski, J. T.; Khare, G. P.; Sidwell, R. W.; Bauer, R. J.; Robins, R. K.; Simon, L. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1174.
(6) Malinoski, F.; Stollar, V. *Virology* **1981**, *110*, 281.
(7) (a) Webster, H. K.; Whaun, J. M. *J. Clin. Invest.* **1982**, *70*, 461.
(8) Hedstrom, L.; Wong, C. C. *Biochemistry* **1990**, *29*, 849.
(9) Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T.; Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256.
(10) Smith, A. R.; Sidwell, R. W.; Robins, R. K. *Ann. Rev. Pharmacol. Toxicol.* **1980**, *20*, 259.
(11) (a) Mizuno, K.; Tsujino, M.; Takada, M.; Hayashi, M.; Atsumi, K.; Asano, K.; Matsuda, T. *J. Antibiot.* **1974**, *27*, 775. (b) Kamata, K.; Okubo, M.; Ishigamori, E.; Masaki, Y.; Uchida, H.; Watanabe, K.; Kashiwagi, N. *Transplantation* **1983**, *35*, 144.
(12) (a) Gebeyehu, G.; Marquez, V. E.; Kelley, J. A.; Cooney, D. A.; Jayaram, H. N.; Johns, D. H. *J. Med. Chem.* **1983**, *26*, 922. (b) Gebeyehu, G.; Marquez, V. E.; Van Cott, A.; Cooney, D. A.; Kelley, J. A.; Jayaram, H. N.; Ahluwalia, G. S.; Dion, R. L.; Wilson, Y. A.; Johns, D. H. *J. Med. Chem.* **1985**, *28*, 99. (c) Yamada, Y.; Natsumeda, Y.; Weber, G. *Biochemistry* **1988**, *27*, 2193.
(13) De Clercq, E.; Luczak, M.; Reepmeyer, J. C.; Kirk, K. L.; Cohen, L. A. *Life Sci* **1975**, *17*, 187.
(14) Sakaguchi, K.; Tsujino, M.; Yoshizawa, M.; Mizuno, K.; Hayano, K. *Cancer Res.* **1975**, *35*, 1643.
(15) Suhadolnik, R. J. In *Nucleosides as Biological Probes*; John Wiley & Sons: New York, 1979; p 56.
(16) Barr, P. J.; Robins, M. J.; Santi, D. V. *Biochemistry* **1983**, *22*, 1696.

0022-2623/91/1834-0778$02.50/0

Chart I



Scheme I[a]



[a] (a) Ac_2O, Et_3N, DMAP, CH_3CN; (b) $(CH_3)_2CH(CH_2)_2ONO$, CH_2I_2; (c) $TMSC{\equiv}CH$, $(PhCN)_2PdCl_2$, Et_3N, CH_3CN; (d) $RC{\equiv}CH$, $(PhCN)_2PdCl_2$, Et_3N, CH_3CN (for **9a**, $Bu_3SnC{\equiv}CTMS$, $(PhCN)_2PdCl_2$, CH_3CN); (e) NH_3/MeOH.

antiallergic and hypotensive activity of 2-alkynyladenosines has been reported by us and it was found that the presence of the triple bond is essential for the activities.[17] 4′-Ethynylthymidine inhibits herpes simplex type 1 induced thymidine kinase.[18] Thus, an alkynyl functional group attached to certain nucleosides would have interesting characteristics. In this paper, we describe the syntheses of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides from AICAR derivatives and their ability to inhibit tumor cell growth in culture.[19] We also include the antineoplastic activity of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6b**, EICAR) against various human tumor cells in vitro and antitumor activity of **6b** against murine P388 and L1210 leukemias in vivo.

Chemistry

The most straightforward synthetic route to the target nucleosides **6** is to introduce an alkynyl group in the 5-position of AICAR (**1**) by using organopalladium chemistry. As our starting material for the palladium-catalyzed cross-coupling reaction with terminal alkynes, the 5-iodo derivative **8** is required. Nucleoside **8** was originally synthesized in poor yield from 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**12**) by the diazotization–substitution reaction with sodium nitrite in the presence of cuprous iodide in fluoroboric acid at −25 °C, followed by hydrolysis of the cyano group.[20] An improved method for synthesizing **8** was found when 2′,3′,5′-tri-*O*-acetyl-AICAR (**7**) was diazotized with isoamyl nitrite in

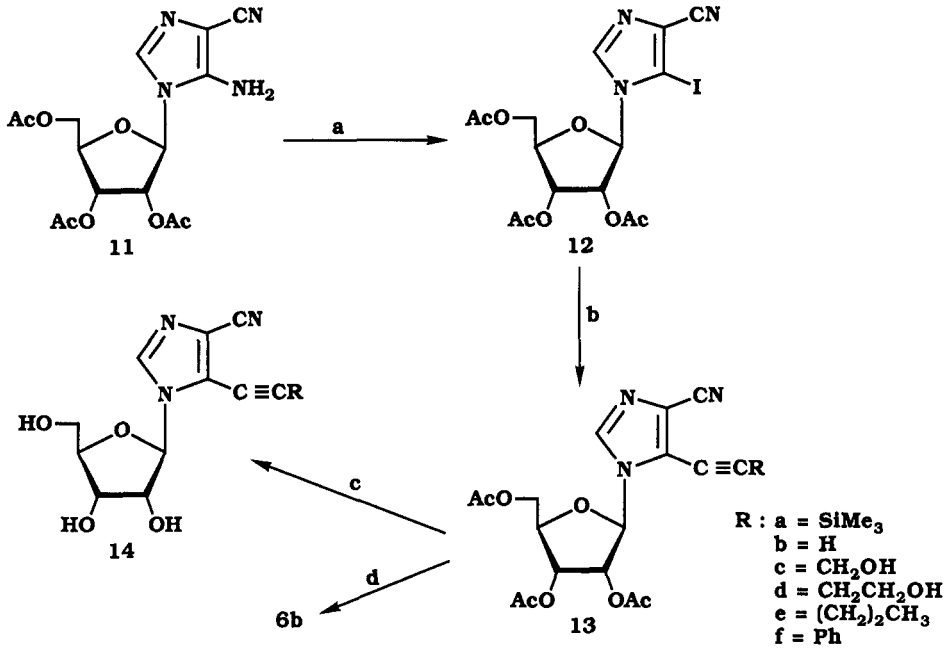
(17) (a) Matsuda, A.; Shinozaki, M.; Miyasaka, T.; Machida, H.; Abiru, T. *Chem. Pharm. Bull.* **1985**, *33*, 1766. (b) Matsuda, A.; Ueda, T. *Nucleosides Nucleotides* **1987**, *6*, 85.

(18) Sharma, R. A.; Bobek, M. *J. Org. Chem.* **1978**, *43*, 367.

(19) Preliminary results: Matsuda, A.; Minakawa, N.; Sasaki, T.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 2730.

(20) Srivastava, P. C.; Streeter, D. G.; Matthews, T. R.; Allen, L. B.; Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1976**, *19*, 1020.

Scheme II[a]



[a] (a) $(CH_3)_2CH(CH_2)_2ONO$, CH_2I_2; (b) RC≡CH, $(PhCN)_2PdCl_2$, Et_3N, CH_3CN; (c) Et_3N/MeOH or NH_3/MeOH; (d) 30% H_2O_2, NH_4OH/MeOH.

diiodomethane at 100 °C; the desired 5-iodo derivative 8 was obtained in 55% yield in two steps from AICAR. This method is much superior to the previous method. In several attempts to improve the yield, dilution of the reaction mixture with acetonitrile or tetrahydrofuran or addition of iodine or cuprous iodide always decreased the yield of 8. Diiodomethane is expensive but after column chromatography it could be recovered.

We next examined the palladium-catalyzed cross-coupling reaction of 8 with propargyl alcohol (Scheme I). When 8 was heated with propargyl alcohol and 10 mol% of palladium catalyst in the presence of triethylamine in a sealed glass tube, the desired 5-(3-hydroxy-1-propyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**9c**) was obtained. As can be seen from Table I, the use of bis(benzonitrile)palladium dichloride in acetonitrile (entry 1) was the best choice of the Pd catalyst as well as the solvent to give **9c** in 94% yield. Use of other Pd catalysts such as bis(triphenylphosphine)palladium dichloride or palladium acetate and other solvents such as *N*,*N*-dimethylformamide (DMF) (entries 3, 4, and 5) reduced the yields of **9c**. Sonogashira et al. found that addition of a catalytic amount of cuprous iodide as cocatalyst to this type of system facilitates the cross-coupling reaction, although the role of copper was not well understood.[21] However, in our reaction, the addition of a catalytic amount of cuprous iodide (entry 2) lowered the yield of **9c**. Under optimized conditions, a series of 5-alkynyl derivatives (**9**) were synthesized in high yields by using phenyl acetylene, 3-butyn-1-ol, 1-pentyne, cyclohexylacetylene, 2-methyl-3-butyn-2-ol, 3-cyclopentyl-1-propyne, 4-phenyl-1-butyne, and 5-cyano-1-pentyne.

In the cross-coupling of 8 with (trimethylsilyl)acetylene, however, the reaction proceeded rather slowly to give a dimer, 1,2-bis(4-carbamoyl-1-β-D-ribofuranosylimidazol-5-yl)acetylene derivative **10a** in 46% yield. During the reaction, desilylation of **9a** by triethylamine afforded **9b**, whose 5-ethynyl group should be more reactive than

Table I. The Cross-Coupling Reaction of 8 with Propargyl Alcohol[a]

entry	catalyst	additive	solvent	time, h	yield of **9c**, %
1	$(PhCN)_2PdCl_2$		CH_3CN	4	94
2	$(PhCN)_2PdCl_2$	CuI	CH_3CN	20	19
3	$(PhCN)_2PdCl_2$		DMF	24	trace
4	$(PPh_3)_2PdCl_2$		CH_3CN	21	42
5	$Pd(OAc)_2$		CH_3CN	23	32

[a] The cross-coupling reaction was done by using 1 mmol of 8 with 10 mol% of Pd catalyst and 1.2 equiv of propargyl alcohol in acetonitrile (5 mL) containing 1.2 equiv of triethylamine in a sealed glass tube at 100 °C under argon. In entry 2, CuI (10 mg, 0.05 mmol) was added.

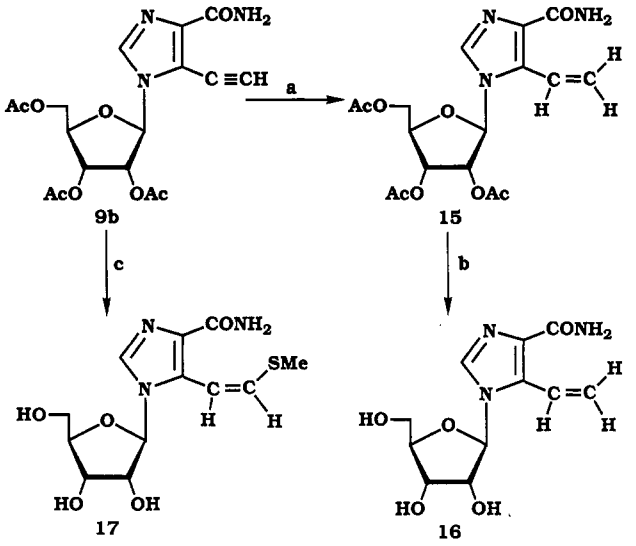
(trimethylsilyl)acetylene itself to couple the remaining 8. To circumvent the undesired dimer formation, trimethyl[(tributylstannyl)ethynyl]silane[22] was used for the coupling reaction with 8 in the absence of triethylamine. Thus, the desired 5-[2-(trimethylsilyl)ethynyl] derivative **9a** was obtained in 77% yield accompanied by a small amount of 5-ethynyl derivative **9b**. Deblocking of these nucleosides **9b–k** by NH_3/MeOH or Et_3N/MeOH gave the target compounds **6b–k** in good yields. Structure of these nucleosides were confirmed by their elemental analyses, mass (MS), and NMR spectroscopic data.

We also examined the cross-coupling reaction of 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**12**) (Scheme II),[20] which was prepared in 70% yield as crystals from the diazotization of **11** with isoamyl nitrite in diiodomethane. Reaction of **12** with terminal acetylenes (phenylacetylene, propargyl alcohol, 3-butyn-1-ol, 1-pentyne, and (trimethylsilyl)acetylene) in the presence of 5 mol% of bis(benzonitrile)palladium dichloride in a mixture of triethylamine and acetonitrile in a sealed glass tube at 100 °C for several hours proceeded smoothly to afford the corresponding 5-alkynyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitriles (**13a,c–f**) in high yields. It is worth noting that the reaction

(21) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1981**, *22*, 421.

(22) Logue, M. W.; Teng, K. *J. Org. Chem.* **1982**, *47*, 2549.

Scheme III[a]



[a] (a) H_2, Pd/C, EtOH; (b) NH_3/MeOH; (c) NaSMe, MeOH.

of (trimethylsilyl)acetylene under these conditions furnished the desired 5-[2-(trimethylsilyl)ethynyl]-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**13a**) in 76% yield without formation of the undesired dimer. Treatment of these nucleosides **13a**, **c–f** with Et_3N/MeOH or NH_3/MeOH gave 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carbonitriles (**14b–f**) in high yields. In the case of **13a**, a trimethylsilyl group was also removed to give the 5-ethynyl derivative **14b**. On the other hand, reaction of **13a** with hydrogen peroxide in NH_4OH/MeOH gave EICAR in 77% yield as crystals.

5-Vinyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**16**) was also prepared by selective catalytic reduction of **9b** using 5% Pd/C as a catalyst, followed by deprotection as usual (Scheme III). The chemical reactivity of the 5-ethynyl group in EICAR was then investigated. Treatment of **9b** with sodium thiomethoxide in MeOH gave (*Z*)-5-[2-(methylthio)vinyl]-1-β-D-ribofuranosylimidazole-4-carboxamide (**17**) in 78% yield.

Antineoplastic Activity

The antineoplastic activity of 5-alkynyl-4-carboxamide derivatives **6b–k**, 5-alkynyl-4-carbonitrile derivatives **14b–f**, and 5-vinyl derivative **16** were evaluated for their ability to inhibit the growth of murine leukemia L1210 cells in culture. The IC_{50} (μg/mL) values for these nucleosides are summarized in Table II. Among these, the most potent inhibitor of cell growth was 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6b**, EICAR). Although an increase of the side chain length of the R in **6c–e** reduced the inhibitory activity, these nucleosides are still more potent than the others. The increase in the size of the R substituent such as phenyl and cyclohexyl groups also reduced or eliminated the antileukemic activity. It is interesting to note that although compounds **6d** and **6e** have similarly sized R substituents, **6d** is 1.8 times more active than **6e**. Also, compound **6g** is 6.8 times less potent than **6c**, but is still more portent than the others having alkyl substituents. From these data, the order of antileukemic potency depends on the size of the R substituents, the smaller the better, and the nature of the R substituents, the more hydrophilic the better. Furthermore, the alkynyl group, especially an ethynyl group at the 5-position, is important for the activity since the 5-vinyl derivative **16** did not show any antileukemic activity up to 100 μg/mL. In a series of the 5-alkynyl-4-cyano derivatives **14b–f**, a similar tendency in the inhibitory activity against L1210 cells was observed but the potency was somewhat reduced than that of the corresponding 4-carboxamide derivatives.

Table II. Inhibitory Effects of 5-Vinyl- and 5-Alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides and -carbonitriles on the Growth of Murine L1210 Cells in Vitro[a]

compd	IC_{50},[b] μg/mL
EICAR	0.18
6c	0.70
6d	1.28
6e	2.29
6f	20.6
6g	4.8
6h	(18.9%)
6i	(−10.7%)
6j	(8.5%)
6k	(3.6%)
14b	1.9
14c	4.1
14d	3.3
14e	14.8
14f	80.3
16	(0.4%)

[a] Cytotoxic activity assay in vitro was done by the method of Carmichael et al.[26] L1210 cells (1×10^4/well) were incubated in the presence or absence of compounds for 72 h. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and o.d. (570 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − o.d. (570 nm) of sample well/o.d. (570 nm) of control well] × 100. [b] IC_{50} (μg/mL) was given as the concentration required for 50% inhibition of cell growth. The number in the parentheses shows the growth inhibition rate at 100 μg/mL.

Table III. Inhibitory Effects of 5-Alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides and Bredinin on the Growth of Various Human Tumor Cell Lines in Vitro[a]

	IC_{50},[b] μg/mL			
cell line	EICAR	**6c**	**6d**	bredinin
PC-3[c]	96	54	>100	>100
PC-6[d]	0.46	ND[l]	12	49
PC-13[e]	86	44	26	>100
Lu-65[f]	3.8	54	15	>100
KB[g]	12	7.5	9.5	>100
SW-480[h]	3.3	3.8	6.2	42
T-24[i]	0.88	8.8	15	>100
OST[j]	28	>100	51	>100
HT-1080[k]	3.0	ND[l]	>100	>100

[a] Each tumor cell line (1×10^4/well) was incubated in the presence or absence of compounds for 96 h (also see Table II and Experimental Section). [b] IC_{50} (μg/mL) was given as the concentration required for 50% inhibition of cell growth. [c] Human lung adenocarcinoma. [d] Human lung small-cell carcinoma. [e] Human large-cell carcinoma. [f] Human giant-cell carcinoma. [g] Human oral epidermoid carcinoma. [h] Human colon adenocarcinoma. [i] Human bladder transitional-cell carcinoma. [j] Human osteosarcoma. [k] Human fibrosarcoma. [l] Not determined.

We next compared EICAR, **6c** and **6d** with a structurally similar naturally occurring nucleoside, bredinin (**4**), the 5′-monophosphate of which is a potent inhibitor of IMP dehydrogenase, as inhibitors of the growth of various human solid tumor cells in culture (Table III). Bredinin is used clinically as an immunosuppressive agent[11] and is also known to inhibit the growth of L5178Y, L, Hela S-3, and RK-13 cells in vitro.[23] These effects are due to the inhibition of IMP dehydrogenase[24] and rather selective to

(23) Sakaguchi, K.; Tsujino, M.; Yoshizawa, M.; Mizuno, K.; Hayano, K. *Cancer Res.* **1975**, *35*, 1643.
(24) Fukui, M.; Inaba, M.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1982**, *42*, 1098.

Table IV. Inhibitory Effects of EICAR and 5-FU on the Growth of Various Mammalian Tumor Cell Lines in Vitro[a]

cell line	IC_{50},[b] μg/mL EICAR	5-FU
CCRF-CEM[c]	1.03	2.10
HL-60[d]	0.23	1.33
K562[e]	0.17	2.20
U937[f]	0.11	0.11
RPMI 8226[g]	0.44	0.07
QG-56[h]	0.18	0.18
Calu-1[i]	0.21	>3.3
QG-90[j]	0.23	0.22
PC-8[k]	0.54	0.42
KU-2[l]	<0.1	0.28
COLO 205[m]	0.11	0.10
HT 1376[n]	0.14	0.58
PANC-1[o]	0.16	0.22
HOS[p]	0.28	0.79
G-292[p]	2.55	>3.3
Saos-2[p]	4.23	0.78

[a] Each tumor cell line (1×10^4/well) was incubated in the presence or absence of compounds for 96 h (also see Table II and Experimental Section). [b] IC_{50} (μg/mL) was given as the concentration required for 50% inhibition of cell growth. [c] Human acute lymphocytic leukemia. [d] Human promyelotic leukemia. [e] Human chronic myelogenous leukemia. [f] Human hystocytic lymphoma. [g] Human myeloma. [h] Human lung squamous-cell carcinoma. [i] Human epidermoid carcinoma. [j] Human lung small-cell carcinoma. [k] Human lung adenocarcinoma. [l] Human renal-cell carcinoma. [m] Human colon adenocarcinoma. [n] Human bladder carcinoma. [o] Human pancreas epitheloid carcinoma. [p] Human osteogenic sarcoma.

lymphocyte cells.[11b] EICAR had potent antitumor activity of PC-6 lung small-cell carcinoma, Lu-65 lung giant-cell carcinoma, SW-480 colon adenocarcinoma, T-24 bladder transitional-cell carcinoma, and HT-1080 fibrosarcoma, but not on PC-3 lung adenocarcinoma, PC-13 lung large-cell carcinoma, and OST osteosarcoma, with IC_{50} values from 0.46 to 3.8 μg/mL. Compounds **6c** and **6d** were less active than EICAR while bredinin was almost inactive up to 100 μg/mL concentrations on this range of solid tumor cells. Whether such differential cytotoxicity between EICAR and bredinin is reflected by different mechanisms of action or to differential susceptibility to phosphorylation of the 5′-hydroxyl group is not clear. The detailed mechanism of action of EICAR will be a future subject.

We further compared the antitumor activity using human tumor cell lines in vitro, other than the above, including leukemias and lymphomas with 5-fluorouracil (5-FU), which is a well-known inhibitor of various solid tumor cells although the mechanism of action may differ from that of EICAR. As can be seen from Table IV, EICAR was cytotoxic to all 16 tumor cells with IC_{50} values from 0.11 to 4.23 μg/mL. Its spectrum of cytotoxicity is quite similar to that of 5-FU.

In vivo antitumor activity of EICAR was also examined against intraperitoneally implanted leukemia L1210 (10^5 cells) or P388 (10^6 cells) in CDF_1 mice, with a dose of 37.5 mg/kg per day intravenously given once a day for 5 days, from the day after tumor transplantation. The antitumor effects of EICAR were estimated by comparison of the median survival time of treated group and that of an untreated group, with 6 CDF_1 female mice in each group. The survival time was greatly increased; the ratio of treated vs the control in median survival time was 148% for L1210 and 135% for P388.[25] Median survival time of the untreated control group was 7.1 days for L1210 (10^5 cells) and 9.4 days for P388 (10^6 cells). Thus, EICAR is an interesting and promising agent that should be considered for further detailed preclinical evaluation.

(25) These in vivo assays were done by Drs. H. Takada, Y. Kashiwazaki, and Y. Yanagi at Sumitomo Pharmaceutical Co., Ltd., to whom our thanks are due.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was YMC gel 60A (70–230 mesh).

5-Iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (8). Triethylamine (11.3 mL, 80 mmol) was added to a suspension of AICAR (1, 5.16 g, 20 mmol) in acetonitrile (50 mL) containing acetic anhydride (7.6 mL, 80 mmol) and DMAP (50 mg).[27] The reaction mixture was stirred for 30 min at room temperature and MeOH (5 mL) was added to the mixture to decompose an excess of acetic anhydride. The mixture was concentrated to dryness in vacuo and the residue was dissolved in $CHCl_3$, which was washed with saturated $NaHCO_3$, followed by H_2O. The separated organic layer was dried (Na_2SO_4) and concentrated to dryness to give crude **7**. Isoamyl nitrite (10 mL) was added to a solution of **7** in diiodomethane (100 mL) at 100 °C (evolution of nitrogen can be observed). After being stirred for 30 min, the reaction mixture was absorbed onto a silica gel column (3.6 × 40 cm), which was washed with $CHCl_3$ to remove diiodomethane (concentration and distillation of this fraction gave pure diiodomethane), and then eluted with 1–4% EtOH in $CHCl_3$. The UV-absorbing fractions were combined, and the solvent was removed to dryness in vacuo to give **8** (5.42 g, 55%) as a bright yellow foam: MS *m/z* 495 (M^+); UV λ_{max} (MeOH) 240 nm (ε 9100); NMR ($CDCl_3$) 7.96 (s, 1 H, H-2), 6.99 (br s, 1 H, $CONH_2$), 5.98 (d, 1 H, H-1′, $J_{1'2'}$ = 4.9 Hz), 5.40 (m, 3 H, H-2′, 3′, $CONH_2$), 4.39 (m, 3 H, H-4′, 5′a, b), 2.17, 2.15, 2.13 (s, each 3 H, acetyl). Anal. ($C_{15}H_{18}IN_3O_8$) C, H, N.

Synthesis of 5-Alkynyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9). Compound **8** (495 mg, 1 mmol), bis(benzonitrile)palladium dichloride (36 mg, 10 mol%), triethylamine (0.16 mL, 1.2 mmol), and terminal alkyne [1.2 equiv of phenyl acetylene, propargyl alcohol, 3-butyn-1-ol, 1-pentyne, cyclohexylacetylene, 2-methyl-3-butyn-2-ol, (trimethylsilyl)acetylene, 3-cyclopentyl-1-propyne, 4-phenyl-1-butyne, or 5-cyano-1-pentyne] in acetonitrile (5 mL) was heated at 100 °C for several hours under an argon atmosphere in a sealed glass tube. After the starting material was completely consumed, judged by TLC, the reaction mixture was filtered through a Celite pad washed with ethanol. The combined filtrate and washings were concentrated to dryness in vacuo and the residue was purified by a silica gel column with appropriately mixed EtOH/$CHCl_3$ solvent system.

5-(Phenylethyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9f). Compound **9f** (370 mg, 79% as a foam) was obtained from **8** with phenylacetylene for 4 h: MS *m/z* 469 (M^+); NMR ($CDCl_3$) 7.76 (s, 1 H, H-2), 7.60 (m, 2 H, Ph), 7.37 (m, 3 H, Ph), 6.96 (br s, 1 H, $CONH_2$), 6.10 (d, 1 H, H-1′, $J_{1'2'}$ = 4.9 Hz), 5.59 (dd, 1 H, H-2′, $J_{1'2'}$ = 4.9, $J_{2'3'}$ = 5.4 Hz), 5.42 (m, 2 H, H-3′, $CONH_2$), 4.40 (m, 3 H, H-4′, 5′a, b), 2.15, 2.13, 2.02 (s, each 3 H, acetyl).

5-(3-Hydroxy-1-propyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9c). Compound **9c** (397 mg, 94% as a foam) was obtained from **8** with propargyl alcohol for 3.5 h: MS *m/z* 423 (M^+); NMR ($CDCl_3$) 7.75 (s, 1 H, H-2), 6.79 (br s, 1 H, $CONH_2$), 5.98 (d, 1 H, H-1′, $J_{1'2'}$ = 4.6 Hz), 5.72 (br s, 1 H, $CONH_2$), 5.65 (dd, 1 H, H-2′, $J_{1'2'}$ = 4.6, $J_{2'3'}$ = 5.4 Hz), 5.40 (dd, 1 H, H-3′, $J_{2'3'}$ = 5.4, $J_{3'4'}$ = 5.1 Hz), 4.57 (br s, 2 H, 5-CH_2OH), 4.40 (m, 3 H, H-4′, 5′a,b), 2.18, 2.15, 2.13 (s, each 3 H, acetyl).

5-(4-Hydroxy-1-butyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9d). From **8** with 3-bu-

tyn-1-ol for 4 h, 405 mg of **9d** was obtained (93% as a foam): MS m/z 437 (M^+); NMR ($CDCl_3$) 7.66 (s, 1 H, H-2), 6.97 (br s, 1 H, $CONH_2$), 5.96 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.62 (dd, 1 H, H-2', $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 5.4 Hz), 5.60 (br s, 1 H, $CONH_2$), 5.43 (dd, 1 H, H-3', $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 4.4 Hz), 4.38 (m, 3 H, H-4', 5'a, b), 3.88 (m, 2 H, 5-CH_2CH_2OH), 2.75 (m, 2 H, 5-CH_2CH_2OH), 2.17, 2.14, 2.12 (s, each 3 H, acetyl).

5-(1-Pentyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9e). From **8** with 1-pentyne for 4 h, 325 mg of **9e** was obtained (75% as a foam): MS m/z 435 (M^+); NMR ($CDCl_3$) 7.68 (s, 1 H, H-2), 6.88 (br s, 1 H, $CONH_2$), 6.02 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.55 (dd, 1 H, H-2', $J_{1',2'}$ = 4.9, $J_{2',3'}$ = 5.4 Hz), 5.47 (br s, 1 H, $CONH_2$), 5.40 (dd, 1 H, H-3', $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 4.4 Hz), 4.38 (m, 3 H, H-4', 5'a,b), 2.54 (m, 2 H, $CH_2CH_2CH_3$), 2.16, 2.11, 2.10 (s, each 3 H, acetyl), 1.79–1.02 (m, 5 H, $CH_2CH_2CH_3$).

5-(Cyclohexylethyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9h). From **8** with cyclohexylacetylene for 16 h, 300 mg (63%) of **9h** was obtained as an oil: MS m/z 475 (M^+); NMR ($CDCl_3$) 7.69 (s, 1 H, H-2), 6.86 (br s, 1 H, $CONH_2$), 6.02 (d, 1 H, H-1', $J_{1',2'}$ = 4.6 Hz), 5.53 (dd, 1 H, H-2', $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 5.1 Hz), 5.39 (m, 2 H, H-3' and $CONH_2$), 4.39 (m, 3 H, H-4', 5'a, b), 2.78 (m, 1 H, cyclohexyl proton), 2.17, 2.11, 2.10 (s, each 3 H, acetyl), 1.56 (m, 10 H, cyclohexyl proton).

5-(3-Hydroxy-3-methyl-1-butyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9g). Compound **9g** was obtained, 430 mg (95%), as an oil by the reaction with 2-methyl-3-butyn-2-ol for 3 h: MS m/z 451 (M^+); NMR ($CDCl_3$) 7.74 (s, 1 H, H-2), 6.94 (br s, 1 H, $CONH_2$), 5.97 (d, 1 H, H-1', $J_{1',2'}$ = 4.4 Hz), 5.73 (br s, 1 H, $CONH_2$), 5.60 (dd, 1 H, H-2', $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 5.1 Hz), 5.40 (dd, 1 H, H-3', $J_{3',2'}$ = 5.1, $J_{3',4'}$ = 5.3 Hz), 4.40 (m, 3 H, H-4', 5'a,b), 2.16, 2.13, 2.11 (s, each 3 H, acetyl), 1.63 (s, 6 H, Me × 2).

5-(3-Cyclopentyl-1-propyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9i). Compound **9i** was obtained, 368 mg (77%), as an oil by the reaction with 3-cyclopentyl-1-propyne for 3 h: MS m/z 475 (M^+); NMR ($CDCl_3$) 7.68 (s, 1 H, H-2), 6.89 (br s, 1 H, $CONH_2$), 6.03 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.53 (dd, 1 H, H-2', $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 5.4 Hz), 5.41 (m, 2 H, H-3' and $CONH_2$), 4.38 (m, 3 H, H-4', 5'a, b), 2.57 (d, 2 H, CH_2-cyclopentyl), 2.16, 2.12, 2.09 (s, each 3 H, acetyl), 1.63 (s, 6 H, cyclopentyl protons).

5-(4-Phenyl-1-butyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9j). Compound **9j** was obtained, 279 mg (56%), as an oil by the reaction with 4-phenyl-1-butyne for 10 h: MS m/z 497 (M^+); NMR ($CDCl_3$) 7.68 (s, 1 H, H-2), 7.29 (m, 5 H, Ph), 6.85 (br s, 1 H, $CONH_2$), 5.89 (d, 1 H, H-1', $J_{1',2'}$ = 4.6 Hz), 5.54 (dd, 1 H, H-2', $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 5.4 Hz), 4.36 (m, 3 H, H-4', 5'a,b), 2.91 (m, 4 H, CH_2CH_2Ph), 2.15, 2.11, 2.07 (s, each 3 H, acetyl).

5-(5-Cyano-1-pentyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9k). Compound **9k** was obtained, 395 mg (86%), as an oil by the reaction with 5-cyano-1-pentyne for 35 h: MS m/z 460 (M^+); NMR ($CDCl_3$) 7.68 (s, 1 H, H-2), 6.89 (br s, 1 H, $CONH_2$), 5.96 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.60 (dd, 1 H, H-2', $J_{2',1'}$ = 4.9, $J_{2',3'}$ = 5.4 Hz), 5.40 (m, 2 H, H-3' and $CONH_2$), 4.38 (m, 3 H, H-4', 5'a,b), 3.14 (m, 2 H, $CH_2CH_2CH_2CN$), 2.74 (m, 2 H, $CH_2CH_2CH_2CN$), 2.15, 2.14, 2.13 (s, each 3 H, acetyl), 1.48 (m, 2 H, $CH_2CH_2CH_2CN$).

These compounds (**9**) obtained the above procedure were used immediately in the final step of the reaction sequence.

5-(Phenylethyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6f). Compound **9f** (360 mg, 0.77 mmol) was dissolved in NH_3/MeOH (saturated at 0 °C, 20 mL), and the mixture was kept overnight at room temperature. The solvent was removed in vacuo, and the residue was purified by a silica gel column (1.8 × 14 cm) eluted with 5–20% EtOH in $CHCl_3$. The UV absorbing fractions were concentrated to dryness. The residue was crystallized from EtOH/Et_2O to give **6f** (198 mg, 75%): mp 168–169 °C; MS m/z 343 (M^+); UV λ_{max} (H_2O) 301 nm (ε 17100), 318 (sh) nm (ε 12800); UV λ_{max} (0.5 N HCl) 294 nm (ε 17000), 310 (sh) nm (ε 13200); UV λ_{max} (0.5 N NaOH) 302 nm (ε 17700), 318 (sh) nm (ε 12400); NMR (DMSO-d_6), 8.19 (s, 1 H, H-2), 7.49 (m, 6 H, Ph and $CONH_2$), 7.24 (br s, 1 H, $CONH_2$), 5.76 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.57 (d, 1 H, 2'-OH, J = 6.1 Hz), 5.23 (d, 1 H, 3'-OH, J = 4.9 Hz), 5.07 (t, 1 H, 5'-OH, J = 5.3 Hz), 4.36 (ddd, 1 H, H-2', $J_{1',2'}$ = 5.1, $J_{2',3'}$ = 4.6 Hz), 4.09 (ddd, 1 H, H-3', $J_{2',3'}$ = 4.6, $J_{3',4'}$ = 4.6 Hz), 3.94 (dt, 1 H, H-4', $J_{3',4'}$ = 4.6, $J_{4',5'}$ = 3.9 Hz), 3.64 (dd, 2 H, H-5'a,b, $J_{4',5'}$ = 3.9 Hz). Anal. ($C_{17}H_{17}N_3O_5 \cdot {}^1/_4H_2O$) C, H, N.

5-(3-Hydroxy-1-propyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6c). In the same manner as for **9f**, treatment of **9c** (300 mg, 0.71 mmol) with NH_3/MeOH (15 mL) gave **6c** as yellow foam, which was crystallized from EtOH/Et_2O to give colorless crystals (180 mg, 86%): mp 148–149 °C; MS m/z 280 (M^+ – OH); UV λ_{max} (H_2O) 267 nm (ε 10600); UV λ_{max} (0.5 N HCl) 255 nm (ε 9700); UV λ_{max} (0.5 N NaOH) 271 nm (ε 11700); NMR (DMSO-d_6) 8.11 (s, 1 H, H-2), 7.31, 7.21 (br s, each 1 H, $CONH_2$), 5.66 (d, 1 H, H-1', $J_{1',2'}$ = 5.4 Hz), 5.50 (d, 1 H, 2'-OH, J = 6.1 Hz), 5.45 (t, 1 H, 5-CH_2OH, J = 6.1 Hz), 5.21 (d, 1 H, 3'-OH, J = 4.9 Hz), 5.04 (t, 1 H, 5'-OH, J = 5.3 Hz), 4.37 (d, 2 H, CH_2OH, J = 6.1 Hz), 4.33 (ddd, 1 H, H-2', $J_{1',2'}$ = 5.4, $J_{2',2'OH}$ = 6.1, $J_{2',3'}$ = 4.4 Hz), 4.07 (ddd, 1 H, H-3', $J_{2',3'}$ = 4.4, $J_{3',3'OH}$ = 4.9, $J_{3',4'}$ = 3.9 Hz), 3.90 (dt, 1 H, H-4', $J_{3',4'}$ = 3.9, $J_{4',5'}$ = 5.9 Hz), 3.57 (dd, 2 H, H-5'a, b, $J_{5',4'}$ = 5.9, $J_{5',5'OH}$ = 5.3 Hz). Anal. ($C_{12}H_{15}N_3O_6$) C, H, N.

5-(4-Hydroxy-1-butyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6d). In the same manner as for **9f**, treatment of **9d** (300 mg, 0.96 mmol) with NH_3/MeOH (15 mL) gave **6d** as yellow foam, which was crystallized from EtOH/Et_2O to give colorless crystals (168 mg, 79%): mp 156–158 °C; MS m/z 311 (M^+); UV λ_{max} (H_2O) 268 nm (ε 11600); UV λ_{max} (0.5 N HCl) 256 nm (ε 10700); UV λ_{max} (0.5 N NaOH) 268 nm (ε 12100); NMR (DMSO-d_6) 8.06 (s, 1 H, H-2), 7.29, 7.08 (br s, each 1 H, $CONH_2$), 5.65 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.51 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 5.9 Hz), 5.18 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 5.1 Hz), 5.00 (m, 2 H, 5'-OH, 5-CH_2OH), 4.25 (ddd, 1 H, H-2', $J_{2',1'}$ = 4.9, $J_{2',2'OH}$ = 5.9, $J_{2',3'}$ = 5.4 Hz), 4.08 (ddd, 1 H, H-3', $J_{3',2'}$ = 5.4, $J_{3',3'OH}$ = 5.1, $J_{3',4'}$ = 4.9 Hz), 3.91 (m, 1 H, H-4'), 3.59 (m, 4 H, H-5'a,b, 5-CH_2OH), 2.64 (m, 2 H, 5-CH_2CH_2OH). Anal. ($C_{13}H_{17}N_3O_6$) C, H, N.

5-(1-Pentyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6e). In the same manner as for **9f**, reaction of **9e** (300 mg, 0.97 mmol) with NH_3/MeOH (15 mL) gave **6e** as a yellow foam which was crystallized from EtOH/hexane to give colorless crystals (171 mg, 80%): mp 172–174 °C; MS m/z 309 (M^+); UV λ_{max} (H_2O) 269 nm (ε 11600); UV λ_{max} (0.5 N HCl) 258 nm (ε 11000); UV λ_{max} (0.5 N NaOH) 269 nm (ε 12100); NMR (DMSO-d_6) 8.06 (s, 1 H, H-2), 7.25, 7.12 (br s, each 1 H, $CONH_2$), 5.65 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.48 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 5.9 Hz), 5.18 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 4.6 Hz), 5.04 (t, 1 H, 5'-OH, $J_{5'OH,5'}$ = 5.3 Hz), 4.30 (ddd, 1 H, H-2', $J_{2',1'}$ = 4.9, $J_{2',2'OH}$ = 5.9, $J_{2',3'}$ = 5.4 Hz), 4.07 (ddd, 1 H, H-3', $J_{3',2'}$ = 5.4, $J_{3',3'OH}$ = 4.6, $J_{3',4'}$ = 4.9 Hz), 3.89 (dt, 1 H, H-4', $J_{4',3'}$ = 4.9, $J_{4',5'}$ = 3.9 Hz), 3.57 (dd, 2 H, H-5'a,b, $J_{5',4'}$ = 3.9, $J_{5',5'OH}$ = 5.3 Hz), 2.42 (m, 2 H, 5-$CH_2CH_2CH_3$), 1.65–0.95 (m, 5 H, 5-$CH_2CH_2CH_3$). Anal. ($C_{14}H_{19}N_3O_5$) C, H, N.

5-(Cyclohexylethyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6h). In the same manner as for **9f**, reaction of **9h** (280 mg, 0.59 mmol) with NH_3/MeOH (15 mL) gave **6h** (168 mg, 82%) as a yellow oil: FABMS m/z 350 (M^+ + 1); UV λ_{max} (H_2O) 270 nm (ε 11800); UV λ_{max} (0.5 N HCl) 259 nm (ε 11200); UV λ_{max} (0.5 N NaOH) 270 nm (ε 11200); NMR (DMSO-d_6 + D_2O) 8.05 (s, 1 H, H-2), 5.64 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 4.27 (t, 1 H, H-2', $J_{2',1'}$ = $J_{2',3'}$ = 4.9 Hz), 4.05 (dd, 1 H, H-3', $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 4.4 Hz), 3.88 (ddd, 1 H, H-4', $J_{4',3'}$ = 4.4, $J_{4',5'}$ = 3.3, $J_{4',5''}$ = 3.8 Hz), 3.61 (dd, 1 H, H-5'a, $J_{5'a,4'}$ = 3.8, $J_{5'a,b}$ = 15.9 Hz), 3.55 (dd, 1 H, H-5'b, $J_{5'b,4'}$ = 3.3, $J_{5'a,b}$ = 15.9 Hz), 2.74 (m, 2 H, cyclohexyl protons), 1.77 (m, 4 H, cyclohexyl protons), 1.48 (m, 2 H, cyclohexyl protons), 1.35 (m, 4 H, cyclohexyl protons). Anal. ($C_{17}H_{23}N_3O_5$) C, H, N.

5-(3-Hydroxy-3-methyl-1-butyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6g). In the same manner as for **9f**, reaction of **9g** (460 mg, 1.02 mmol) with NH_3/MeOH (10 mL) gave **6g** (256 mg, 77%) as a pale yellow foam: FABMS m/z 326 (M^+ + 1); UV λ_{max} (H_2O) 267 nm (ε 7900); UV λ_{max} (0.5 N HCl) 256 nm (ε 7400); UV λ_{max} (0.5 N NaOH) 270 nm (ε 7600); NMR (DMSO-d_6 + D_2O) 8.09 (s, 1 H, H-2), 5.63 (d, 1 H, H-1', $J_{1',2'}$ = 5.0 Hz), 4.25 (t, 1 H, H-2', $J_{2',1'}$ = $J_{2',3'}$ = 5.0 Hz), 4.06 (dd, 1 H, H-3', $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 4.3 Hz), 3.90 (dt, 1 H, H-4', $J_{4',3'}$ = 4.3, $J_{4',5'}$ = 3.8 Hz), 3.62 (dd, 2 H, H-5'a, b, $J_{5',4'}$ = 3.8, $J_{5'a,b}$ = 12.1 Hz), 1.47 (s, 6 H, Me × 2). Anal. ($C_{14}H_{19}N_3O_6$) C, H, N.

5-(3-Cyclopentyl-1-propyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6i). In the same manner as for **9f**, reaction of **9i** (360 mg, 0.76 mmol) with NH_3/MeOH (10 mL) gave **6i** (204 mg, 77%) as a yellow oil: FABMS m/z 350 ($M^+ + 1$); UV λ_{max} (H_2O) 269 nm (ε 11300); UV λ_{max} (0.5 N HCl) 259 nm (ε 10600); UV λ_{max} (0.5 N NaOH) 271 nm (ε 10700); NMR (DMSO-d_6 + D_2O) 8.05 (s, 1 H, H-2), 5.64 (d, 1 H, H-1′, $J_{1',2'}$ = 5.5 Hz), 4.28 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.5, $J_{2',3'}$ = 5.0 Hz), 4.05 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 4.4 Hz), 3.88 (ddd, 1 H, H-4′, $J_{4',3'}$ = 4.4, $J_{4',5'a}$ = 3.3, $J_{4',5'b}$ = 3.8 Hz), 3.55 (m, 2 H, H-5′a,b), 2.07 (m, 2 H, CH_2-c-C_5H_9), 1.56 (m, 9 H, cyclopentyl protons). Anal. ($C_{17}H_{23}N_3O_5$) C, H, N.

5-(4-Phenyl-1-butyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6j). In the same manner as for **9f**, reaction of **9j** (274 mg, 0.55 mmol) with MH_3/MeOH (15 mL) gave **6j** (179 mg, 88%) as a pale brown foam: FABMS m/z 372 ($M^+ + 1$); UV λ_{max} (H_2O) 268 nm (ε 7400); UV λ_{max} (0.5 N HCl) 257 nm (ε 6400); UV λ_{max} (0.5 N NaOH) 268 nm (ε 6000); NMR (DMSO-d_6 + D_2O) 8.05 (s, 1 H, H-2), 7.32 (m, 5 H, Ph), 5.63 (d, 1 H, H-1′, $J_{1',2'}$ = 5.5 Hz), 4.28 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.5, $J_{2',3'}$ = 5.0 Hz), 4.05 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 4.4 Hz), 3.92 (dt, 1 H, H-4′, $J_{4',3'}$ = 4.4, $J_{4',5'}$ = 3.8 Hz), 3.56 (m, 2 H, H-5′a, b), 2.80 (m, 4 H, CH_2CH_2Ph). Anal. ($C_{19}H_{21}N_3O_5$) C, H, N.

5-(5-Cyano-1-pentyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carboxamide (6k). In the same manner as for **9f**, reaction of **9k** (250 mg, 0.54 mmol) with NH_3/MeOH (15 mL) gave **6k** (119 mg, 66%) as a pale yellow foam: FABMS m/z 355 ($M^+ + 1$); UV λ_{max} (H_2O) 268 nm (ε 11200); UV λ_{max} (0.5 N HCl) 256 nm (ε 10700); UV λ_{max} (0.5 N NaOH) 269 nm (ε 10900); NMR (DMSO-d_6 + D_2O) 8.06 (s, 1 H, H-2), 5.63 (d, 1 H, H-1′, $J_{1',2'}$ = 5.0 Hz), 4.27 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.0, $J_{2',3'}$ = 5.5 Hz), 4.04 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 4.4 Hz), 3.90 (dt, 1 H, H-4′, $J_{4',3'}$ = 4.4, $J_{4',5'}$ = 3.9 Hz), 3.61 (dd, 1 H, H-5′a, $J_{5'a,4'}$ = 3.9, $J_{5'a,b}$ = 11.5 Hz), 3.56 (dd, 1 H, H-5′b, $J_{5'b,4'}$ = 3.9, $J_{5'a,b}$ = 11.5 Hz), 2.80 (t, 2 H, $CH_2CH_2CH_2CN$), 2.63 (t, 2 H, $CH_2CH_2CH_2CN$), 1.87 (dt, 2 H, $CH_2CH_2CH_2CN$). Anal. ($C_{15}H_{18}N_4O_5$) C, H, N.

1,2-Bis[4-carbamoyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazol-5-yl]acetylene (10a). A mixture of 8 (495 mg, 1 mmol), bis(benzonitrile)palladium dichloride (18 mg, 5 mol%), Et_3N (0.16 mL, 1.2 equiv), (trimethylsilyl)acetylene (0.17 mL, 1.2 equiv) in acetonitrile (5 mL) was heated under argon in a sealed glass tube at 100 °C for 22 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated to dryness. The residue was purified by a silica gel column (2.7 × 17 cm) eluted with 0–12% EtOH in $CHCl_3$. The fractions containing **10a** were concentrated to dryness to give 208 mg (55%) as a brown oil: MS m/z 760 (M^+); NMR ($CDCl_3$) 7.81 (s, 2 H, H-2 × 2), 6.99 (br s, 2 H, $CONH_2$ × 2), 6.30 (d, 2 H, H-1′ × 2, $J_{1',2'}$ = 4.2 Hz), 5.96 (br s, 2 H, $CONH_2$), 5.68 (m, 2 H, H-2′ × 2), 5.37 (m, 2 H, H-3′ × 2), 4.40 (m, 6 H, H-4′ × 2, 5′a, b × 2), 2.13 (m, 18 H, acetyl × 6).

1,2-Bis(4-carbamoyl-1-β-D-ribofuranosylimidazol-5-yl)acetylene (10b). Compound **10a** (150 mg, 0.19 mmol) was dissolved in NH_3/MeOH (20 mL), and the mixture was kept at room temperature overnight. The solvent was removed in vacuo, and the residue was crystallized from H_2O to give **10b** (36.5 mg, 36%): mp 203–206 °C; FABMS m/z 509 ($M^+ + 1$); UV λ_{max} (H_2O) 320 nm (ε 18800), 340 nm (sh) (ε 13400); UV λ_{max} (0.5 N HCl) 316 nm (ε 20800), 316 nm (sh) (ε 15200); UV λ_{max} (0.5 N NaOH) 317 nm (ε 19300), 340 nm (sh) (ε 11900); NMR (DMSO-d_6) 8.29 (s, 2 H, H-2 × 2), 7.72 (br s, 2 H, $CONH_2$ × 2), 7.56 (br s, 2 H, $CONH_2$ × 2), 5.86 (d, 2 H, H-1′ × 2, $J_{1',2'}$ = 3.3 Hz), 5.71 (br d, 2 H, 2′-OH × 2), 5.15 (br s, 2 H, 3′-OH × 2), 5.01 (br s, 2 H, 5′-OH × 2), 4.29 (br s, 2 H, H-2′ × 2), 4.10 (br s, 2 H, H-3′ × 2), 3.98 (m, 2 H, H-4′ × 2), 3.75 (br d, 2 H, H-5′a × 2), 3.60 (br d, 2 H, H-5′b × 2). Anal. ($C_{20}H_{24}N_6O_{10}\cdot{}^1/_5H_2O$) C, H, N.

5-[2-(Trimethylsilyl)-1-ethyn-1-yl]-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (9a). A mixture of 8 (990 mg, 2 mmol), bis(benzonitrile) palladium dichloride (36 mg, 5 mol%), trimethyl[(tributylstannyl)ethnyl]silane[22] in acetonitrile (7 mL) in a sealed glass tube was heated under argon at 100 °C for 17 h. After the usual workup, purification by a silica gel column gave **9a** (668 mg, 72%): MS m/z 465 (M^+); NMR ($CDCl_3$) 7.73 (s, 1 H, H-2), 6.92 (br s, 1 H, $CONH_2$), 6.03 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 5.52 (dd, 1 H, H-2′, $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 5.4 Hz), 5.42 (m, 2 H, H-3′, $CONH_2$), 4.40 (m, 3 H, H-4′,5′a,b), 2.17, 2.11, 2.10 (s, each 3 H, acetyl), 0.30 (s, 9 H, trimethylsilyl).

5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (6b). (a) Compound **9a** (400 mg, 0.86 mmol) was treated with NH_3/MeOH (saturated at 0 °C, 25 mL) at room temperature overnight. The solvent was concentrated in vacuo, and the residue was purified by a silica gel column (2.3 × 15 cm) eluted with 5–30% EtOH in $CHCl_3$. The fractions containing **6b** were concentrated to dryness. The residue was crystallized from EtOH to give **6b** (200 mg, 87%): mp 182–185 °C; MS m/z 267 (M^+); UV λ_{max} (H_2O) 269 nm (ε 9800); UV λ_{max} (0.5 N NaOH) 263 nm (ε 10300); NMR (DMSO-d_6), 8.13 (s, 1 H, H-2), 7.34, 7.24 (br s, each 1 H, $CONH_2$), 5.66 (d, 1 H, H-1′, $J_{1',2'}$ = 5.4 Hz), 5.52 (d, 1 H, 2′-OH, $J_{2',2'\text{-OH}}$ = 6.1 Hz), 5.20 (d, 1 H, 3′-OH, $J_{3',3'\text{-OH}}$ = 4.9 Hz), 5.05 (t, 1 H, 5′-OH, $J_{5',5'\text{-OH}}$ = 5.3 Hz), 4.86 (s, 1 H, 5-acetylene proton), 4.32 (ddd, 1 H, H-2′, $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 4.9, $J_{2',2'\text{OH}}$ = 6.1 Hz), 4.06 (ddd, 1 H, H-3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 3.9, $J_{3',3'\text{OH}}$ = 4.9 Hz), 3.92 (dt, 1 H, H-4′, $J_{4',3'}$ = 3.9, $J_{4',5'}$ = 3.9 Hz), 3.57 (dd, 2 H, H-5′a, b, $J_{5',4'}$ = 3.9, $J_{5',5'\text{OH}}$ = 5.3 Hz). Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

(b) Hydrogen peroxide (0.5 mL, 30%) was added to a solution of compound **13a** (289 mg, 0.65 mmol) in aqueous NH_4OH/MeOH (8 mL, 1:1 v/v). The reaction mixture was stirred for 45 min at room temperature. The solvent was concentrated to dryness in vacuo, and the residue was purified by a silica gel column (2.3 × 7 cm) eluted with 5–30% EtOH in $CHCl_3$. The fractions containing **6b** were concentrated to dryness to give 133 mg (77%) as crystals. An analytical sample was obtained by recrystallization from EtOH.

5-Iodo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (12). AICAR (10.32 g, 40 mmol) was acetylated as described in the synthesis of **8** to give the crude **7**, which was coevaporated with anhydrous pyridine (2 × 20 mL). A solution of the crude **7** was added to *p*-toluenesulfonyl chloride (9.15 g, 48 mmol). The reaction mixture was stirred for 2 h at room temperature, and the solvent was removed in vacuo. The residue was purified by a silica gel column (3.6 × 38 cm), eluted with 0–4% EtOH in $CHCl_3$, to give 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**11**, 13.63 g, 93% as a foam): MS m/z 366 (M^+); NMR ($CDCl_3$) 7.27 (s, 1 H, H-2), 5.66 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 5.44 (dd, 1 H, H-2′, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 5.4 Hz), 5.28 (m, 1 H, H-3′), 4.71 (br s, 2 H, 5-NH_2), 4.42 (m, 3 H, H-4′, 5′a,b), 2.15 (s, 6 H, acetyl), 2.14 (s, 3 H, acetyl). Compound **11** (10.88 g, 29.7 mmol) was dissolved in diiodomethane (150 mL) at 100 °C. Isoamyl nitrite (15 mL) was added to the above solution. After being stirred for 30 min, the reaction mixture was adsorbed onto a silica gel column (3.6 × 40 cm) which was washed with $CHCl_3$ to remove diiodomethane and then eluted with 1–4% EtOH in $CHCl_3$. The fractions containing **12** were evaporated in vacuo, and the residue was crystallized from EtOH to give 9.86 g (70%): mp 139–141 °C (ref.[20] mp 140–141 °C); MS m/z 477 (M^+); UV λ_{max} 236 nm (ε 9400); NMR ($CDCl_3$) 7.98 (s, 1 H, H-2), 5.84 (d, 1 H, H-1′, $J_{1',2'}$ = 4.9 Hz), 5.49 (dd, 1H, H-2′, $J_{2',1'}$ = 4.9, $J_{2',3'}$ = 5.4 Hz), 5.38 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 3.9 Hz), 4.39 (m, 3 H, H-4′,5′a,b), 2.15 (s, 9 H, acetyl × 3). Anal. ($C_{15}H_{16}IN_3O_7$) C, H, N.

Synthesis of 5-Alkynyl-1-(2,3,5-tri-O-acetyl-1-β-D-ribofuranosyl)imidazole-4-carbonitrile (13a,c–f). A mixture of **12** (477 mg, 1 mmol), bis(benzonitrile)palladium dichloride (18 mg, 5 mol%), terminal alkyne [1.2 equiv of phenyl acetylene, propargyl alcohol, 3-butyn-1-ol, 1-pentyne, or (trimethylsilyl)-acetylene] in acetonitrile (5 mL) containing triethylamine (0.16 mL, 1.2 mmol) was heated in a sealed tube at 100 °C for several hours under argon atmosphere. The reaction mixture was filtered through a Celite pad and washed with EtOH. The combined filtrate and washings were concentrated to dryness in vacuo. The residue obtained was purified by a silica gel column.

5-(Phenylethyn-1-yl)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13f). From **12** with phenylacetylene for 3.5 h, 438 mg (97% as a foam) of **13f** was obtained: MS m/z 451 (M^+); NMR ($CDCl_3$) 7.82 (s, 1 H, H-2), 7.49 (m, 5 H, Ph), 6.01 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6 Hz), 5.60 (dd, 1 H, H-2′, $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 5.6 Hz), 4.40 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 4.6 Hz), 4.39 (m, 3 H, H-4′,5′a,b), 2.14, 2.13, 2.07 (s, each 3 H, acetyl).

5-(3-Hydroxy-1-propyn-1-yl)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13c). Compound **13c** was obtained in 77% yield (311 mg as a foam) by the reaction

with propargyl alcohol for 2 h: MS m/z 405 (M^+); NMR ($CDCl_3$) 7.79 (s, 1 H, H-2), 5.88 (d, 1 H, H-1', $J_{1',2'}$ = 3.6 Hz), 5.70 (dd, 1 H, H-2', $J_{2',1'}$ = 3.7, $J_{2',3'}$ = 5.4 Hz), 5.40 (dd, 1 H, H-3', $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 5.6 Hz), 4.58 (d, 2 H, 5-CH_2OH, J = 6.6 Hz), 4.40 (m, 3 H, H-4',5'a,b), 2.68 (t, 1 H, 5-CH_2OH, J = 6.6 Hz), 2.14 (s, 9 H, acetyl × 3).

5-(4-Hydroxy-1-butyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13d). Compound **13d** was obtained in 79% yield (331 mg as a foam) by the reaction with 3-butyn-1-ol for 5.5 h: MS m/z 419 (M^+); NMR ($CDCl_3$) 7.73 (s, 1 H, H-2), 5.89 (d, 1 H, H-1', $J_{1',2'}$ = 4.4 Hz), 5.66 (dd, 1 H, H-2', $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 5.4 Hz), 5.37 (dd, 1 H, H-3', $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 5.1 Hz), 4.38 (m, 3 H, H-4', H-5'a, b), 3.86 (dt, 2 H, 5-CH_2CH_2OH, J = 5.9, J = 6.4 Hz), 2.81 (t, 2 H, 5-CH_2CH_2OH, J = 5.9 Hz), 2.33 (t, 1 H, 5-CH_2CH_2OH, J = 6.4 Hz), 2.13 (s, 9 H, acetyl × 3).

5-(1-Pentyn-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13e). Compound **13e** was obtained in 72% yield (302 mg as a foam) by the reaction with 1-pentyne for 3.5 h: MS m/z 417 (M^+); NMR ($CDCl_3$) 7.74 (s, 1 H, H-2), 5.91 (d, 1 H, H-1', $J_{1',2'}$ = 4.6 Hz), 5.55 (dd, 1 H, H-2', $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 5.1 Hz), 5.41 (dd, 1 H, H-3', $J_{3',2'}$ = 5.1, $J_{3',4'}$ = 4.4 Hz), 4.38 (m, 3 H, H-4',5'a,b), 5.23 (t, 2 H, 5-C$H_2CH_2CH_3$, J = 6.4 Hz), 2.14 (s, 3 H, acetyl), 2.12 (s, 6 H, acetyl × 2), 1.61 (dt, 2 H, 5-CH_2CH_2CH_3, J = 6.4, J = 7.6 Hz), 1.08 (t, 3 H, 5-CH_2CH_2CH_3, J = 7.6 Hz).

5-[2-(Trimethylsilyl)-1-ethyn-1-yl]-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13a). Compound **13a** was obtained in 76% yield (341 mg as a foam) by the reaction with (trimethylsilyl)acetylene for 5 h: MS m/z 447 (M^+); NMR ($CDCl_3$) 7.78 (s, 1 H, H-2), 5.92 (d, 1 H, H-1', $J_{1',2'}$ = 4.4 Hz), 5.53 (dd, 1 H, H-2', $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 5.1 Hz), 5.37 (dd, 1 H, H-3', $J_{3',2'}$ = 5.1, $J_{3',4'}$ = 4.9 Hz), 4.40 (m, 3 H, H-4', 5'a,b), 2.12 (s, 9 H, acetyl × 3), 0.30 (s, 9 H, trimethyl). Anal. ($C_{20}H_{25}N_3O_7Si$) C, H, N.

These compounds (**13a–e**) obtained by the above procedure were used immediately in the final step of the reaction sequence.

5-(Phenylethyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carbonitrile (14f). A solution of **13f** (400 mg, 0.88 mmol) in a mixture of MeOH (10 mL) and triethylamine (1 mL) was stirred at room temperature overnight. The solvent was removed by evaporation, and the residue was purified by a silica gel column (2.7 × 9 cm) eluted with 5–15% EtOH in $CHCl_3$. The fractions containing **14f** were concentrated to dryness. The residue was crystallized from EtOH/hexane to give 229 mg (79%): mp 187–188 °C; MS m/z 325 (M^+); UV λ_{max} (H_2O) 296 nm (ε 18 800), 315 nm (ε 13 600); UV λ_{max} (0.5 N HCl) 296 nm (ε 18 100), 315 nm (ε 13 500); UV λ_{max} (0.5 N NaOH) 296 nm (ε 18 100), 315 nm (ε 13 000); NMR (DMSO-d_6) 8.39 (s, 1 H, H-2), 7.60 (m, 5 H, Ph), 5.77 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.67 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 6.1 Hz), 5.30 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 4.9 Hz), 5.12 (t, 1 H, 5'-OH, $J_{5'OH,5'}$ = 5.3 Hz), 4.37 (ddd, 1 H, H-2', $J_{2',1'}$ = 4.9, $J_{2',2'OH}$ = 6.1, $J_{2',3'}$ = 5.1 Hz), 4.04 (ddd, 1 H, H-3', $J_{3',2'}$ = 5.1, $J_{3',3'OH}$ = 4.9, $J_{3',4'}$ = 4.6 Hz), 3.99 (dt, 1 H, H-4', $J_{4',3'}$ = 4.6, $J_{4',5'}$ = 3.4 Hz), 3.63 (dd, 2 H, H-5'a, b, $J_{5',4'}$ = 3.4, $J_{5',5'OH}$ = 5.3 Hz). Anal. ($C_{17}H_{15}N_3O_4$) C, H, N.

5-(3-Hydroxy-1-propyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carbonitrile (14c). Treatment of **13c** (250 mg, 0.61 mmol) in a mixture of MeOH and triethylamine gave **14c** (146 mg, 85%, triturated with ether) as pale red crystals: mp 138–139 °C; MS m/z 279 (M^+); UV λ_{max} (H_2O) 259 nm (ε 12 900), 272 nm (sh) (ε 10 400); UV λ_{max} (0.5 N HCl) 259 nm (ε 14 400), 271 nm (sh) (ε 11 100); UV λ_{max} (0.5 N NaOH) 263 nm (ε 13 300), 275 nm (sh) (ε 10 900); NMR (DMSO-d_6) 8.32 (s, 1 H, H-2), 5.64 (d, 1 H, H-1', $J_{1',2'}$ = 5.4 Hz), 5.61 (t, 1 H, 5-CH_2OH, J = 6.1 Hz), 5.60 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 6.1 Hz), 5.27 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 4.9 Hz), 5.07 (t, 1 H, 5'-OH, $J_{5'OH,5'}$ = 5.3 Hz), 4.43 (d, 2 H, 5-CH_2OH, J = 6.1 Hz), 4.35 (m, 1 H, H-2'), 4.03 (m, 2 H, H-3',4'), 3.58 (m, 2 H, H-5'a,b). Anal. ($C_{12}H_{13}N_3O_5$) C, H, N.

5-(4-Hydroxy-1-butyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carbonitrile (14d). Reaction of **13d** (320 mg, 0.76 mmol) in MeOH and triethylamine gave **14d** (167 mg, 75%) as a pale yellow oil: MS m/z 293 (M^+); UV λ_{max} (H_2O) 259 nm (ε 13 100), 270 nm (sh) (ε 10 600); UV λ_{max} (0.5 N HCl) 259 nm (ε 13 100), 271 nm (sh) (ε 9900); UV λ_{max} (0.5 N NaOH) 260 nm (ε 13 300), 272 nm (sh) (ε 10 800); NMR (DMSO-d_6) 8.27 (s, 1 H, H-2), 5.64 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.60 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 6.2 Hz), 5.26 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 5.1 Hz), 5.09 (dd, 1 H, 5'-OH, $J_{5'OH,5'}$ = 5.1, $J_{5'OH,5''}$ = 5.5 Hz), 5.04 (t, 1 H, 5-CH_2OH, J = 5.5 Hz), 4.32 (ddd, 1 H, H-2', $J_{2',1'}$ = 5.1, $J_{2',2'OH}$ = 6.2, $J_{2',3'}$ = 4.8 Hz), 4.06 (ddd, 1 H, H-3', $J_{3',2'}$ = 4.8, $J_{3',3'OH}$ = 5.1, $J_{3',4'}$ = 4.4 Hz), 3.93 (ddd, 1 H, H-4', $J_{4',3'}$ = 4.4, $J_{4',5'a}$ = 4.0, $J_{4',5'b}$ 3.7 Hz), 3.61 (m, 4 H, H-5'a,b, 5-CH_2CH_2OH), 2.73 (t, 2 H, 5-CH_2CH_2OH, J = 6.6 Hz). Anal. ($C_{13}H_{15}N_3O_5 \cdot {}^1/_3H_2O$) C, H, N.

5-(1-Pentyn-1-yl)-1-β-D-ribofuranosylimidazole-4-carbonitrile (14e). Treatment of **13e** (302 mg, 0.72 mmol) in MeOH and triethylamine gave a yellow foam. This was recrystallized from *i*-PrOH/hexane to give **14e** (186 mg, 88%): mp 156–157 °C; MS m/z 291 (M^+); UV λ_{max} (H_2O) 259 nm (ε 13 800), 272 nm (sh) (ε 11 200); UV λ_{max} (0.5 N HCl) 259 nm (ε 13 400); UV λ_{max} (0.5 N NaOH) 261 nm (ε 13 700), 2.72 nm (sh) (ε 11 200); NMR (DMSO-d_6) 8.28 (s, 1 H, H-2), 5.62 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.59 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 5.9 Hz), 5.26 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 5.1 Hz), 5.09 (dd, 1 H, 5'-OH, $J_{5'OH,5'a}$ = 5.1, $J_{5'OH,5'b}$ = 5.5 Hz), 4.33 (ddd, 1 H, H-2', $J_{2',1'}$ = 5.1, $J_{2',2'OH}$ = 5.9, $J_{2',3'}$ = 4.8 Hz), 4.06 (ddd, 1 H, H-3', $J_{3',2'}$ = 4.8, $J_{3',3'OH}$ = 5.1, $J_{3',4'}$ = 4.4 Hz), 3.92 (ddd, 1 H, H-4', $J_{4',3'}$ = 4.4, $J_{4',5'a}$ = 4.0, $J_{4',5'b}$ = 3.7 Hz), 3.61 (ddd, 1 H, H-5'a, $J_{5'a,4'}$ = 4.0, $J_{5'a,b}$ = 12.1, $J_{5'a,5'OH}$ = 5.1 Hz), 5.52 (ddd, 1 H, H-5'b, $J_{5'b,4'}$ = 3.7, $J_{5'a,b}$ = 12.1, $J_{5'b,5'OH}$ = 5.5 Hz), 2.58 (t, 2 H, C$H_2CH_2CH_3$, J = 7.0 Hz), 1.60 (dt, 2 H, 5-CH_2CH_2CH_3, J = 7.0, J = 7.3 Hz), 1.03 (t, 3 H, 5-CH_2CH_2CH_3, J = 7.3 Hz). Anal. ($C_{14}H_{17}N_3O_4 \cdot {}^1/_5H_2O$) C, H, N.

5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carbonitrile (14b). Compound **13a** (618 mg, 1.38 mmol) was dissolved in NH_3/MeOH (saturated at 0 °C, 20 mL) and the mixture was kept at room temperature overnight. The solvent was removed in vacuo and the residue was purified by a silica gel column (2.7 × 12 cm) eluted with 5–30% EtOH in $CHCl_3$. The fractions containing **14b** were concentrated to dryness to give a crude **14b**. From the NMR spectrum, it was found that the residue contains acetamide. The residue was purified by a HPLC (YMC-D-ODS-5, flow 9 mL/min, retention time 17 min) with 30% MeOH–H_2O as eluent. The fractions containing **14b** were concentrated to dryness to give 220 mg (64%) as a brown oil: MS m/z 249 (M^+); UV λ_{max} (H_2O) 255 nm (ε 10 200), 266 nm (sh) (ε 8000); UV λ_{max} (0.5 N HCl) 255 nm (ε 10 400), 267 nm (sh) (ε 7900); UV λ_{max} (0.5 N NaOH) 256 nm (ε 10 000), 268 nm (sh) (ε 7800); NMR (DMSO-d_6) 8.33 (s, 1 H, H-2), 5.63 (d, 1 H, H-2', $J_{1',2'}$ = 5.1 Hz), 5.60 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 5.9 Hz), 5.35 (s, 1 H, acetylene proton), 5.25 (d, 1 H, 3'-OH, $J_{3'OH,3'}$ = 4.9 Hz), 5.07 (t, 1 H, 5'-OH, $J_{5'OH,5'}$ = 5.3 Hz), 4.31 (ddd, 1 H, H-2', $J_{2',1'}$ = 5.1, $J_{2',2'OH}$ = 5.9, $J_{2',3'}$ = 5.4 Hz), 4.05 (ddd, 1 H, H-3', $J_{3',2'}$ = 5.4, $J_{3',3'OH}$ = 4.9, $J_{3',4'}$ = 3.7 Hz), 3.94 (dt, 1 H, H-4', $J_{4',3'}$ = 3.7, $J_{4',5'}$ = 3.9 Hz), 3.56 (dd, 2 H, H-5'a, b, $J_{5',4'}$ = 3.9, $J_{5',5'OH}$ = 5.3 Hz). Anal. ($C_{11}H_{11}N_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

5-Vinyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxamide (15). A suspension of **6b** (267 mg, 1 mmol), DMAP (5 mg), Et_3N (0.56 mL, 4 mmol), and acetic anhydride (0.38 mL, 4 mmol) in acetonitrile (10 mL) was stirred for 3 h at room temperature. The solvent was removed in vacuo, and the resulting oil was partitioned between EtOAc and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness to give **9b** (345 mg, 90%, as a white foam). A suspension of **9b** (280 mg, 0.71 mmol), 5% Pd/C (30 mg) in EtOH (50 mL) was shaken under hydrogen (50 psi) on a Parr hydrogenation apparatus at room temperature for 12 h. The reaction mixture was filtered through a Celite pad, further washed with EtOH, and then the filtrate and washings were concentrated in vacuo. The residue was purified by a silica gel column (1.8 × 11 cm) eluted with hexane/AcOEt (1:1–1:3). The UV absorbing fractions were evaporated to dryness to give **15** (214 mg, 76%) as a foam: MS m/z 395 (M^+); NMR ($CDCl_3$) 7.75 (s, 1 H, H-2), 7.21 (1 H, 5-CH=CH_aH$_b$, J = 18.3, J = 12.0 Hz), 7.05 (br s, 1 H, $CONH_2$), 5.96 (d, 1 H, H-1', $J_{1',2'}$ = 5.4 Hz), 5.83 (dd, 1 H, 5-CH_c=CH_aH$_b$, $J_{a,c}$ = 18.3, $J_{a,b}$ = 1.0 Hz), 5.59 (dd, 1 H, H-2', $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 6.1 Hz), 5.47 (dd, 1 H, 5-CH_c=CH_aH$_b$, $J_{b,c}$ = 12.0, $J_{a,b}$ = 1.0 Hz), 5.43 (m, 2 H, H-3', $CONH_2$), 4.36 (m, 3 H, H-4',5'a,b), 2.16, 2.14, 2.09 (s, each 3 H, acetyl).

5-Vinyl-1-β-D-ribofuranosylimidazole-4-carboxamide (16). A solution of **15** (80 mg, 0.20 mmol) in NH_3/MeOH (5 mL) was kept overnight at room temperature. The solvent was removed in vacuo, and the residue was purified by a silica gel column (1.8 × 6 cm) with 5–30% EtOH in $CHCl_3$ as eluent. The UV absorbing fractions were combined, and the solvent was concentrated to

dryness to give **16** (46 mg, 85%). The solid was recrystallized from EtOH-Et_2O: mp 193 °C (sintered from 100 °C); MS m/z 269 (M^+); UV λ_{max} (H_2O) 248 nm (ϵ 7300); UV λ_{max} (0.5 N NaOH) 249 nm (ϵ 9800); NMR (DMSO-d_6 + D_2O) 8.13 (s, 1 H, H-2), 7.20 (dd, 1 H, 5-C*H*=CH_2, J = 18.3, J = 12.2 Hz), 5.86 (dd, 1 H, 5-CH_c=C$H_a$$H_b$, $J_{a,b}$ = 1.5, $J_{a,c}$ = 18.3 Hz), 5.64 (d, 1 H, H-1′, $J_{1',2'}$ = 5.1 Hz), 5.50 (dd, 1 H, 5-CH_c=$CH_a$$H_b$, $J_{a,b}$ = 1.5, $J_{b,c}$ = 12.2 Hz), 4.31 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 4.9 Hz), 4.08 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 4.4 Hz), 3.92 (m, 1 H, H-4′), 3.60 (m, 2 H, H-5′a,b). Anal. ($C_{11}H_{15}N_3O_5$) C, H, N.

(*Z*)-5-[2-(Methylthio)vinyl]-1-β-D-ribofuranosylimidazole-4-carboxamide (17). A solution of sodium thiomethoxide (15% in H_2O, 1 mL) was added to a solution of **9b** (270 mg, 1.01 mmol) in MeOH (15 mL). The mixture was stirred for 7 h at room temperature. The mixture was neutralized with 1 N HCl and concentrated in vacuo. The residue was passed through a short silica gel column (1.5 × 2 cm) to remove the salt. The final purification was done by a HPLC (Inertsil-ODS, 20.0 × 250 mm, flow 9 mL/min) eluted with 20% MeOH in H_2O. The fractions having retention time at 8 min were collected, and the solvent was removed in vacuo to give **17** (248 mg, 78%) as a white foam: FABMS m/z 316 (M^+ + 1); UV λ_{max} (H_2O) 237 nm (ϵ 11 100), 281 nm (ϵ 4800); UV λ_{max} (0.5 N HCl) 286 nm (ϵ 3800); UV λ_{max} (0.5 N NaOH) 240 nm (ϵ 10 200), 283 nm (ϵ 4800); NMR (DMSO-d_6 + D_2O) 8.01 (s, 1 H, H-2), 6.63 (d, 1 H, 5-C*H*=CH, J = 10.4 Hz), 6.34 (d, 1 H, 5-CH=C*H*, J = 10.4 Hz), 5.48 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 4.06 (dd, 1 H, H-2′, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 4.9 Hz), 4.03 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 3.8 Hz), 3.87 (dt, 1 H, H-4′, $J_{4',3'}$ = 3.8, $J_{4',5'}$ = 3.3 Hz), 3.59 (m, 2 H, H-5′a,b), 2.25 (s, 3 H, SC*H*$_3$). Anal. ($C_{12}H_{17}N_3O_5S \cdot {}^1/_5H_2O$) C, H, N, S.

Assay of in Vitro Antitumor Activity. In vitro antitumor activity was determined by using murine and/or human tumor cells. Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. Tumor cells (1×10^4 cells/mL) were cultured in a CO_2 gas incubator at 37 °C for 72 h (or for 96 h for Table III and IV) in 1 mL of medium containing various concentrations of test compound. Their viability, estimated by use of a variation of a colorimetric [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay,[26] was compared to that of control cells incubated in the identical medium without the compound. The antitumor activity evaluated as IC_{50} (the concentration in μg/mL required for 50% inhibition of cell growth). The IC_{50} value was obtained by plotting the logarithm of concentration of the test compound vs the growth rate (percentage of control) of the treated cells. The results are representative of three separate experiments.

Acknowledgment. This investigation was supported in part by Grants-in-Aid for Developmental Scientific Research, Cancer Research, and Special Project Research on Cancer-Bioscience from the Ministry of Education, Science, and Culture of Japan.

Registry No. 1, 2627-69-2; **6a**, 131194-97-3; **6b**, 118908-07-9; **6c**, 118908-02-4; **6d**, 118908-04-6; **6e**, 118908-05-7; **6f**, 118908-03-5; **6g**, 131195-02-3; **6h**, 131195-04-5; **6i**, 131195-06-7; **6j**, 131195-08-9; **6k**, 131195-10-3; **8**, 118744-90-4; **9a**, 118908-01-3; **9b**, 126004-24-8; **9c**, 118934-03-5; **9d**, 118907-98-5; **9e**, 118907-99-6; **9f**, 118907-97-4; **9g**, 131195-01-2; **9h**, 131195-03-4; **9i**, 131195-05-6; **9j**, 131195-07-8; **9k**, 131195-09-0; **10a**, 118907-96-3; **10b**, 131195-00-1; **11**, 23192-63-4; **12**, 59354-00-6; **13a**, 126004-21-5; **13b**, 131195-12-5; **13c**, 126004-19-1; **13d**, 131195-11-4; **13e**, 131195-13-6; **13f**, 126004-18-0; **14a**, 131194-98-4; **14b**, 126004-13-5; **14c**, 126004-14-6; **14d**, 126004-15-7; **14e**, 131195-14-7; **14f**, 126004-22-6; **15**, 114485-26-6; **16**, 114485-11-9; **17**, 131194-99-5.

(26) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
(27) Matsuda, A.; Shinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. *Synthesis* **1986**, 385.

Structure–Activity Relationship of Mutagenic Aromatic and Heteroaromatic Nitro Compounds. Correlation with Molecular Orbital Energies and Hydrophobicity

Asim Kumar Debnath,[†] Rosa L. Lopez de Compadre,[§] Gargi Debnath,[†] Alan J. Shusterman,[‡] and Corwin Hansch*[,†]

Department of Chemistry, Pomona College, Claremont, California 91711, Department of Chemistry, Reed College, Portland, Oregon 97202-8199, and Department of Biopharmaceutical Science, College of Pharmacy, University of Arkansas for Medical Science, Little Rock, Arkansas 72205. *Received August 27, 1990*

A review of the literature yielded data on over 200 aromatic and heteroaromatic nitro compounds tested for mutagenicity in the Ames test using *S. typhimurium* TA98. From the data, a quantitative structure–activity relationship (QSAR) has been derived for 188 congeners. The main determinants of mutagenicity are the hydrophobicity (modeled by octanol/water partition coefficients) and the energies of the lowest unoccupied molecular orbitals calculated using the AM1 method. It is also shown that chemicals possessing three or more fused rings possess much greater mutagenic potency than compounds with one or two fused rings. Since the QSAR is based on a very wide range in structural variation, aromatic rings from benzene to coronene are included as well as many different types of heterocycles, it is a significant step toward a predictive toxicology of value in the design of less mutagenic bioactive compounds.

Introduction

The problem of toxicity in drug development becomes of ever greater importance as more sophisticated methods of epidemiology uncover more subtle forms of toxicity. Concern has shifted from acute toxicity to that resulting from long term exposure to drugs and/or their metabolic products. Such potential toxicity, when identified early in drug development, can avoid needless expense and loss of time. Kapeghian and Traina[1] point out in their review of experimental toxicology in the pharmaceutical industry that the time has come to move from "descriptive toxicology" to "predictive toxicology". This advice applies not only to medicinal chemistry and to the production of all industrial chemicals, pesticides, solvents, etc., but also to the recognition of dangers inherent in so called "natural environmental" compounds which may even be present in common foods. In this report we consider a rather general QSAR for correlating the mutagenicity of aromatic and heteroaromatic nitro compounds. Despite the fact that aromatic nitro compounds have been found to be both mutagenic and carcinogenic,[2-5] drugs containing this

[†] Pomona College.
[‡] Reed College.
[§] University of Arkansas for Medical Science.

(1) Kapeghian, J. C.; Traina, V. M. *Med. Res. Rev.* **1990**, *10*, 271.
(2) Xu, X. B.; Nachtman, D. P.; Jin, Z. L.; Rapport, S. M.; Burlingam, A. L. *Anal. Chim. Acta* **1982**, 136; 163.
(3) Rosenkranz, H. S.; Mermelstein, R. J. J. *Environ. Sci. Health* **1985**, *C3*, 221.

0022-2623/91/1834-0786$02.50/0