Improved Synthesis and Antitumor Activity of 1-Deazaadenosine

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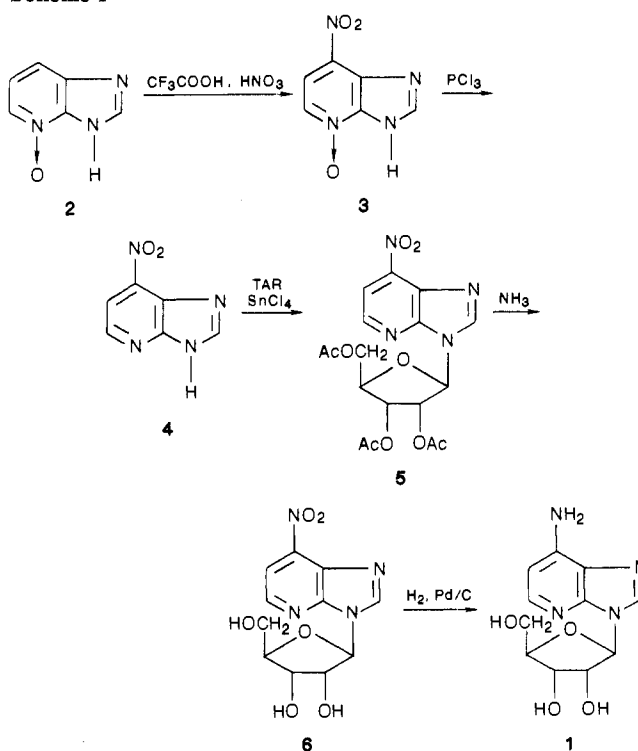
A more convenient synthetic route to 1-deazaadenosine (1) by reduction of the new nucleoside 7-nitro-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (6) is reported. Compound 6 was obtained by reaction of 7-nitroimidazo[4,5-b]pyridine with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of stannic chloride followed by treatment with methanolic ammonia. 1-Deazaadenosine (1) showed good activity in vitro as inhibitor of HeLa, KB, P388, and L1210 leukemia cell line growth, with ID$_{50}$ values ranging from 0.34 μM (KB) to 1.8 μM (P388). The nitro derivative 6 demonstrated moderate activity against the same cell lines.

The adenosine analogue 1-deazaadenosine (1) was found to have a wide spectrum of biological properties. Recently our studies stressed its activity as an inhibitor of blood platelet aggregation[1] and of adenosine deaminase[2] and as an agonist of the adenosine receptors.[3] Furthermore, Japanese authors have reported, without providing experimental data, that 1 shows potent antileukemic activity.[4] Several syntheses of 1 have been reported[5] and the most convenient one appears to be that described by Itoh and co-workers, who have obtained in six steps the title compound in 25% overall yield, starting from imidazo[4,5-b]pyridine 4-oxide (2).[5d] Recently we reported an improved synthesis of 7-nitroimidazo[4,5-b]pyridine 4-oxide (3) accomplished by nitration of 2 with a nitric acid–trifluoroacetic acid mixture.[1] The possibility that such a compound might be an useful intermediate in the synthesis of 1-deazaadenosine prompted us to design a new synthetic route to 1.

Chemistry

The synthesis of 1-deazaadenosine (1) was carried out according to Scheme I. Reaction of compound 3 with phosphorus trichloride in acetonitrile gave the deoxygenated derivative 4, which was condensed with an equimolar

Scheme I

amount of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (TAR) in acetonitrile in the presence of a catalytic amount of stannic chloride to provide 7-nitro-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-3H-imidazo[4,5-b]pyridine (5) in 82% yield. The 7-amino-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (1-deazaadenosine, 1) was easily obtained from the deblocked nucleoside 6 in 93% yield by hydrogenation

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Table I. In Vitro Antitumor Activity of 1-Deazaadenosine (1) and the Nitro Compound 6[a]

	ID_{50},[b] μM						
	human cell lines				murine cell lines		
	HeLa		KB		P388	P388/DX[c]	L1210
compd	72 h	144 h	72 h	144 h	72 h	72 h	72 h
1	1.2	1.2	0.3	0.6	1.1	0.4	1.8
6	64.1	27.0	33.8	13.1	12.5	8.1	11.8
5-FU	6.1	0.8	13.8	2.0	0.3	0.1	0.4
ara-C	0.6	0.1	1.0	1.1	0.02	0.01	0.02
ara-A	46.0	27.7	89.8	25.4	12.7	10.8	23.6

[a] 5×10^5 exponentially growing cells were exposed to varying concentration of drug for 72–144 h. The cells were then counted with a Coulter counter.[6] [b] Inhibitory dose 50 (Id_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. [c] Doxorubicin-resistant cell line.

Table II. Antitumor Activity of 1-Deazaadenosine (1) and the Nitro Derivative 6 against Leukemia P388/DX and L1210 Cells[a]

| | P388/DX | | | L1210 | | |
compd	dose, mg/kg per day	% T/C[b]	toxic deaths[c]	dose, mg/kg per day	% T/C[b]	toxic deaths[c]
1	3.1	106	0/10	6.3	100	0/10
	6.3	106	0/10	12.5	113	0/10
	12.5	111	0/10	25.0	88	6/10
6	100.0	95	0/10	75.0	106	0/10
	150.0	97	2/20	150.0	106	0/10
	300.0	22	8/20			

[a] BDF$_1$ mice were inoculated ip with P388 leukemia cells (10^6 cells/mouse) and CDF$_1$ mice were inoculated ip with L1210 leukemia cells (10^5 cells/mouse), respectively. Treatments (ip) were inititated 24 h after tumor inoculation; drugs were dissolved in distilled water just before use and administrated daily, in a volume of 10 mL/kg of body weight, for 7 days (compound 1) and for 9 days (compound 6). [b] Median survival time of treated mice/median survival time of controls $\times$ 100; significant effect 125. [c] Number of mice died for toxicity/number of treated mice. Toxicity was evaluated on the basis of the gross autopsy findings, mainly from the reduction of spleen and liver size.

with Pd/C at 50 psi. Compound 1 exhibited identical physical properties with that previously reported.[5] The present route for the synthesis of 1-deazaadenosine, with an overall yield of about 40% based on 2, involves less steps and represents an improvement in yield and procedure with respect to that described by Itoh and co-workers.[5d]

Biological Results

1-Deazaadenosine (1) and the nitro compound 6 were tested in vitro for their cytotoxic activity against two human cell lines (HeLa and KB) and three murine leukemia cell lines (P388, P388 doxorubicin resistant, and L1210) and compared with that of 5-fluorouracil (5-FU), 1-β-D-arabinofuranosylcytosine (*ara*-C) and 1-β-D-arabino-furanosyladenine (*ara*-A) (Table I). 1-Deazaadenosine (1) showed good activity against all the tumor cells investigated and was found to be a much better inhibitor of cell growth than *ara*-A. Its cytostatic activity is also comparable to that of 5-FU in all the cell lines and to that of *ara*-C in the human cell lines. 1-Deazaadenosine is however significantly less active than *ara*-C against murine leukemia P388 and L1210. The nitro compound 6 still exhibited moderate activity mostly against KB and P388 leukemia cells.

Compounds 1 and 6 were tested in vivo against leukemia P388/DX and L1210 in the mouse. The results, given in Table II, showed that both compounds are inactive. A possible explanation for the inactivity of 1-deazaadenosine in the in vivo assays might be found in the chemical instability of this molecule. In fact, we observed that 1-deazaadenosine is not a substrate of adenosine deaminase, of which it is instead an inhibitor,[2] but undergoes hydrolytic cleavage of the ribose moiety more easily than adenosine.[7] An alternative explanation might be poor

selectivity of 1-deazaadenosine for P388 and L1210 cells over normal cells in mice.

Experimental Section

Synthesis. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer, with tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. TLC were carried out on precoated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are indicated by atomic symbols and are within ±0.4% of theoretical values.

7-Nitroimidazo[4,5-*b*]pyridine (4). To a solution of 8.0 g (44.4 mmol) of 7-nitroimidazo[4,5-*b*]pyridine 4-oxide (3)[1] in 150 mL of dry acetonitrile was added dropwise 34 mL of phosphorus trichloride and the mixture was heated at 80 °C for 2 h. After cooling, a yellow solid precipitated, which was collected by filtration and washed with ether and then with saturated sodium carbonate solution. Recrystallization from water provided 5.7 g (82%) of 4 as yellow needles: mp 219–222 °C dec; ^{1}H NMR (Me$_2$SO-d_6) δ 8.05 (d, 1, $J_{5,6}$ = 5.5 Hz, H-5), 8.75 (d, 1, $J_{6,5}$ = 5.5 Hz, H-6), 8.9 (s, 1, H-2), 11.14 (br s, 1, NH exchangeable with D$_2$O). Anal. (C$_6$H$_4$N$_4$O$_2$) C, H, N.

7-Nitro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (5). To a mixture of 5.0 g (15.0 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (TAR) in 150 mL of dry acetonitrile and 2.5 g (15.0 mmol) of 4 was added dropwise 3.7 mL of freshly distilled stannic chloride and the solution was stirred at room temperature for 7 h. The reaction mixture was neutralized with 15 g of NaHCO$_3$ in 150 mL of H$_2$O and then extracted several times with chloroform. The organic layers were dried (Na$_2$SO$_4$) and evaporated, and the residue was chromatographed on a silica gel column eluting with CHCl$_3$–C$_6$H$_6$–MeOH (80:10:10) to give 5.2 g (82%) of 5 as a viscous pure solid: IR ν 1535 cm^{-1} (NO$_2$); ^{1}H NMR (Me$_2$SO-d_6) δ 2.03, 2.06, 2.15 (s, 9, three COCH$_3$), 4.40 (m, 3, H-4' and CH$_2$-5'), 5.71 (m, 1, H-3'), 6.12 (m, 1, *H*-2'), 6.50 (d, 1, J = 4.5 Hz, H-1'), 8.09 (d, 1, $J_{5,6}$ = 5 Hz, H-5), 8.75 (d, 1, $J_{6,5}$ = 5 Hz, H-6), 9.07 (s, 1, H-2). Anal. (C$_{17}$H$_{18}$N$_4$O$_9$) C, H, N.

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7-Nitro-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (6). A solution of 4.5 g (10.6 mmol) of **5** in 135 mL of methanol saturated at 0 °C with ammonia was set aside at room temperature for 24 h. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with EtOAc–MeOH (85:15) to give 2.7 g (86%) of **6** as a chromatographically pure solid: mp 142–146 °C dec; IR ν 1530 cm^{-1} (NO$_2$); ^{1}H NMR (Me$_2$SO-d_6) δ 3.68 (m, 2, CH$_2$-5′), 4.03 (m, 1, H-4′), 4.25 (m, 1, H-3′), 4.65 (m, 1, H-2′), 6.20 (d, 1, J = 5.4 Hz, H-1′) 8.05 (d, 1, $J_{5,6}$ = 5.4 Hz, H-5), 8.72 (d, 1, $J_{6,5}$ = 5.4 Hz, H-6), 9.12 (s, 1, H-2). Anal. (C$_{11}$H$_{12}$N$_4$O$_6$) C, H, N.

7-Amino-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (1-Deazaadenosine) (1). To a solution of 2.6 g (8.2 mmol) of **6** in 300 mL of MeOH was added 1.3 g of 10% Pd/C, and the mixture was shaken with hydrogen at 50 psi for 0.5 h. The catalyst was removed by filtration and the filtrate was evaporated to give 2.03 g (93%) of **1** as a chromatographically pure solid: mp 262–263 °C dec (lit.[5a] mp 263–264 °C).

Biological Studies. Cell Lines. The two human cell lines HeLa (human cervix carcinoma) and KB (human oral epidermid carcinoma) were routinely maintained in monolayer culture in minimum essential medium (Eagle) with nonessential amino acids and Earles' BSS, supplemented with 10% fetal calf serum and antibiotics (50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL kanamycin). The three murine leukemia cell lines (P388, P388/DX, and L1210) were established in vitro as suspension cultures and maintained in APMI 1640 medium supplemented with 10% fetal calf serum, 20 μM 2-mercaptoethanol, 2 μM L-glutamine, and antibiotics. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO$_2$. The characteristics and the in vitro culture of these cell lines have previously been described.[8-10]

Antitumor Activity in Vivo. Animals and Tumors. Inbred DBA/2 and first generation hybrid (C57B1/6 × DBA/2)F$_1$ (BDF$_1$) and (Balb/c × DBA/2)F$_1$ (CDF$_1$) adult mice of both sexes were used for the evaluation of antitumor activity. All animals were obtained from Charles River Italia (Calco, Como, Italy). The animals were 2–3 months old, weighed 20–24 g, and were kept under standard laboratory conditions. The subline of P388 leukemia resistant to DX (P388/DX) was obtained by repeated exposure to the drug in Dr. F. M. Schabel's laboratory (Southern Research Institute, Birmingham, AL) and maintained in our facilities in BDF$_1$ mice given weekly ip passages of 10^7 cells/6 mg/kg ip of doxorubicin. For experimental purposes, 10^6 cells/mouse were transplanted ip in the same strain of animals. Ascitic L1210 leukemia was maintained by serial ip passages in DBA/2 mice, according to Geran et al.[9] For experimental purposes, 10^5 cells/mouse were inoculated ip in CDF$_1$ mice.

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Anticoagulant Peptides: Nature of the Interaction of the C-Terminal Region of Hirudin with a Noncatalytic Binding Site on Thrombin

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A series of 20 C-terminal fragment analogues of the anticoagulant peptide hirudin were synthesized by solid-phase techniques in order to investigate the nature of the thrombin–hirudin interaction. Inhibition of plasma fibrin clot formation by thrombin in vitro was used as a measure of anticoagulant activity. In the minimum region necessary for detectable anticoagulant activity, hirudin$_{56-64}$, positions Phe^{56}, Glu^{57}, Ile^{59}, Pro^{60}, and Leu^{64} are sensitive to modification. These residues are apparently important for direct interaction with thrombin or for maintaining a favorable conformation for the interaction. On the basis of conformational analysis of this region by computational methods, a "kinked" amphipathic α-helical structure, which orients all of the residues most critical for activity on one face of the helix, is proposed.

Hirudin is a 65 amino acid anticoagulant peptide produced in the salivary glands of the medicinal leech (*Hirudo medicinalis*). It binds tightly to thrombin ($K_d \simeq 10^{-11}$ M) thereby inhibiting the cleavage of fibrinogen and subsequent fibrin clot formation.[1,2] Hirudin was originally isolated and named by Jacoby[3] in 1904 and purified to homogeneity and characterized in 1957 by Markwardt.[4] The amino acid sequence and covalent structure have been completed by Dodt et al. (Figure 1).[5,6] In addition, the nature of the thrombin–hirudin interaction has been partially characterized by semisynthetic modification of

thrombin[1] and hirudin.[8] These studies indicate that the amino groups of thrombin and the carboxyl groups of hirudin are important for their interaction. Kinetic studies of the thrombin–hirudin complex at varying ionic strengths by Stone and Hofsteenge[2] suggest that the initial and rate-limiting interaction between hirudin and thrombin is diffusion controlled and possibly ionic in nature. Additionally, they noted that this initial interaction takes place at a site distinct from the catalytic site of the enzyme although hirudin additionally binds to the catalytic site. Chang[9] noted a reverse homology of the C-terminal 20 amino acids of hirudin to fibrinopeptides A and B. This coupled with semisynthetic C-terminal modifications of hirudin suggests that this region of hirudin might be in-

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