Purine and 8-Substituted Purine Arabinofuranosyl and Ribofuranosyl Nucleoside Derivatives as Potential Inducers of the Differentiation of the Friend Erythroleukemia

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Several antimetabolites have been demonstrated to have the capacity to initiate differentiation in vitro of a variety of leukemic cell lines. To explore the structural requirements for this activity, a series of purine and 8-substituted purine arabinofuranosyl and ribofuranosyl nucleoside derivatives were synthesized and tested as inducers of the differentiation of Friend murine erythroleukemia cells. 9-(β-D-Arabinofuranosyl)hypoxanthine and 6-(hydroxyamino)-9-(β-D-arabinofuranosyl)purine were effective inducers of maturation, producing 82% and 74% benzidine-positive cells, a measure of the number of cells synthesizing hemoglobin. 6-Mercapto-9-(β-D-ribofuranosyl)purine and 6-(methylmercapto)-9-(β-D-ribofuranosyl)purine and their corresponding β-D-arabinofuranosyl derivatives were also effective initiators of maturation, causing approximately 50% of the cell population to assume a differentiated phenotype.

The chemotherapeutic agents currently in use for the treatment of the leukemias function by the cytodestruction of the neoplastic cells. This approach produces significant morbidity. Thus, alternate approaches that do not involve cell kill are sorely needed. One such approach, which is based upon the concept that the leukemias represent diseases of blocked maturation, is to use agents that produce terminal differentiation of leukemic cells by converting them to end-stage forms with no proliferative capacity. The Friend murine erythroleukemia and HL-60 human promyelocytic leukemia represent model systems that undergo both morphological and functional maturation after exposure to a large number of chemicals including polar solvents,[2] hormones,[3] vitamins,[4,5] tumor promotors,[6] and antineoplastic agents.[7,8] Among the chemotherapeutic agents that initiate maturation are the 6-thiopurines, 6-thioguanine and 6-mercaptopurine, which are at best only weak inducers of differentiation in Friend and HL-60 leukemia cells.[9-11] These purine antimetabolites, however, are exceedingly effective inducers of differentiation in variants of both of these leukemias that lack hypoxanthine–guanine phosphoribosyltransferase (HGPRT) activity.[10,12-14] Our laboratory has characterized the metabolites of 6-thioguanine formed by parental and HGPRT-negative HL-60 cells, demonstrating that the free base 6-thioguanine itself and its deoxyribonucleoside are responsible for inducing differentiation, whereas the formation of 6-thioguanosine 5′-phosphate is essential for the production of cytotoxicity by this purine antimetabolite.[14] These findings led us to synthesize a variety of 8-substituted guanosine and 2′-deoxyguanosine derivatives that might be poor substrates or nonsubstrates for potential nucleotide-forming enzymes for evaluation as inducers of the differentiation of the Friend murine erythroleukemia.[15] The most active agents developed in the guanosine series were 8-substituted $N(CH_3)_2$, $NHCH_3$, NH_2, OH, and SO_2CH_3, which produced 68, 42, 34, 33, and 30% benzidine-positive (hemoglobin-containing) cells at concentrations of 5, 1, 0.4, 5, and 5 mM, respectively. The 8-OH derivative of the 2′-deoxyguanosine series produced comparable activity, causing 62% benzidine-positive cells at a level of 0.2 mM. These findings support the concept that 8-substituted analogues of guanosine and 2′-deoxyguanosine have the potential to terminate leukemic cell proliferation through conversion to end-stage differentiated cells. On the basis of these findings, we have fabricated a variety of purines and 8-substituted purine arabinofuranosyl and ribofuranosyl nucleoside analogues that were expected to be ineffective substrates for purine nucleoside phosphorylase and HGPRT and have measured their toxicities and capacity to induce the erythroid differentiation of Friend erythroleukemia cells.

Chemistry

Various purine and 8-substituted purine arabinofuranosyl and ribofuranosyl nucleoside analogues (Figure 1) have been synthesized and tested as potential inducers of the differentiation of the Friend erythroleukemia. Treatment of 9-(β-D-arabinofuranosyl)adenine (**1**, ara-A) with nitrous acid gave 9-(β-D-arabinofuranosyl)hypoxanthine (**2**). Acetylation of **2** generated the triacetate, which was treated with phosphorus oxychloride[16] followed by ethanolic hydroxylamine at room temperature for 5 days, to afford the 6-hydroxylamino derivative **3**.[17] Oxidation of 8-bromo-9-(β-D-arabinofuranosyl)adenine (**4**) with sodium nitrite and acetic acid yielded 8-bromo-9-(β-D-arabinofuranosyl)hypoxanthine (**5**). Nucleophilic dis-

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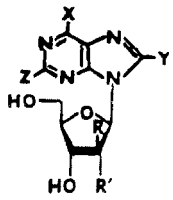

1: R = OH, R′ = H, X = NH_2, Y = H, Z = H
2: R = OH, R′ = H, X = OH, Y = H, Z = H
3: R = OH, R′ = H, X = NHOH, Y = H, Z = H
4: R = OH, R′ = H, X = NH_2, Y = Br, Z = H
5: R = OH, R′ = H, X = OH, Y = Br, Z = H
6: R = OH, R′ = H, X = OH, Y = N_3, Z = H
7: R = OH, R′ = H, X = OH, Y = NH_2, Z = H
8: R = OH, R′ = H, X = OH, Y = $N(CH_3)_2$, Z = H
9: R = N_3, R′ = H, X = NH_2, Y = H, Z = H
10: R = N_3, R′ = H, X = OH, Y = H, Z = H
11: R = OH, R′ = H, X = SH, Y = H, Z = H
12: R = OH, R′ = H, X = SCH_3, Y = H, Z = H
13: R = H, R′ = OH, X = NH_2, Y = H, Z = H
14: R = H, R′ = OH, X = NH_2, Y = Br, Z = H
15: R = H, R′ = OH, X = OH, Y = H, Z = H
16: R = H, R′ = OH, X = OH, Y = Br, Z = H
17: R = H, R′ = OH, X = OH, Y = N_3, Z = H
18: R = H, R′ = OH, X = OH, Y = NH_2, Z = H
19: R = H, R′ = OH, X = OH, Y = $N(CH_3)_2$, Z = H
20: R = H, R′ = OH, X = OH, Y = SH, Z = H
21: R = H, R′ = OH, X = SH, Y = H, Z = H
22: R = H, R′ = OH, X = SCH_3, Y = H, Z = H
23: R = H, R′ = OH, X = OH, Y = H, Z = OH
24: R = H, R′ = OH, X = OH, Y = Br, Z = OH
25: R = H, R′ = OH, X = OH, Y = $OCH_2C_6H_5$, Z = OH
26: R = H, R′ = OH, X = OH, Y = OH, Z = OH

Figure 1. Structures of purine and 8-substituted purine arabinofuranosyl and ribofuranosyl nucleoside derivatives.

placement of the 8-bromo group in **5** by lithium azide and dimethylamine afforded the respective 8-azido and 8-dimethylamino derivatives **6** and **8**. The 8-amino analogue **7** was obtained by catalytic hydrogenation (10% Pd/C) of **6** in EtOH–H_2O. 9-(2-Azido-2-deoxy-β-D-arabinofuranosyl)hypoxanthine (**10**) was synthesized by nitrous acid oxidation of 9-(2-azido-2-deoxy-β-D-arabinofuranosyl)adenine (**9**).[18] 6-Mercapto-9-(β-D-arabinofuranosyl)purine (**11**) and 6-(methylmercapto)-9-(β-D-arabinofuranosyl)purine (**12**) were fabricated from 9-(β-D-2,3,5-tri-*O*-acetylarabinofuranosyl)hypoxanthine by the methodology of Reist et al.[16] 8-Bromoadenosine (**14**) was oxidized with sodium nitrite in acetic acid to 8-bromoxanthosine (**16**) by the procedure of Holmes et al.[19] Subsequent treatment of **16** with lithium azide, dimethylamine, and thiourea produced the corresponding 8-azido (**17**), 8-dimethylamino (**19**), and 8-mercapto (**20**) derivatives. The 8-amino analogue **18** was prepared by catalytic hydrogenation (10% Pd/C) of **17** in EtOH–H_2O. The 6-mercapto and 6-methylmercapto derivatives **21** and **22** were obtained from inosine by methodology similar to that employed for the synthesis of compounds **11** and **12**.[16] The 8-bromo and 8-(benzyloxy)xanthosine derivatives, **24** and **25**, were readily synthesized by treatment of 8-bromoguanosine and 8-(benzyloxy)guanosine with sodium nitrite and glacial acetic acid.[19,20] Debenzylation of **25** by catalytic hydrogenation (10% Pd/C) yielded the 8-hydroxy analogue **26**.

Biological Results

Compounds were evaluated by their capacity to produce cytotoxicity and to induce differentiation. Cytotoxicity was expressed as the concentration required for 50% inhibition of growth, and differentiation was expressed as the maximum percentage of benzidine-positive cells produced at the most effective concentration of the agent that caused maturation. Since adenosine-like derivatives were potential substrates for adenosine deaminase, they were tested in the presence and absence of a 25 μM concentration of the adenosine deaminase inhibitor deoxycoformycin. Deoxycoformycin at this concentration was nontoxic to Friend leukemia cells and did not serve as an inducer of differentiation. Two interesting findings were observed:

First, 9-(β-D-arabinofuranosyl)hypoxanthine (**2**, Ara-Ino) and 6-(hydroxylamino)-9-(β-D-arabinofuranosyl)purine (**3**) were equally potent as initiators of maturation requiring 0.2 mM concentration for optimal induction of differentiation. Deoxycoformycin increased the cytotoxicity of **3** by 8-fold and prevented this agent from initiating differentiation. 9-(β-D-Arabinofuranosyl)adenine (Ara-A), which is converted to **2** in the absence of deoxycoformycin, was 7 times more cytotoxic to Friend erythroleukemia cells than **2**. Ara-A was slightly active in inducing hemoglobin synthesis in the presence of deoxycoformycin but inactive in the absence of the adenosine deaminase inhibitor. These findings indicate that ara-Ino (**2**) is an active inducer of differentiation and that **3** functions to induce differentiation by forming ara-Ino (**2**). Ara-A did not induce differentiation even though it can be converted to ara-Ino (**2**). The inactivity of ara-A as an inducer of maturation derives from an inability to achieve the dosage levels required to initiate commitment without major toxicity. Although the cytotoxicities of 8-substituted derivatives of **2** were much lower than that of **2**, they were ineffective in initiating maturation except for the 8-azido derivative **6**, which was partially active in inducing differentiation. 9-(2-Azido-2-deoxy-β-D-arabinofuranosyl)hypoxanthine (**10**) was an active inducer of differentiation, and its cytotoxicity was considerably less than that of ara-Ino (**2**). It would appear that the mechanism of induction produced by ara-Ino (**2**) is not mediated through the liberation of the active initiator of maturation hypoxanthine by purine nucleoside phosphorylase, since the concentration of hypoxanthine required for the induction of differentiation in Friend erythroleukemia cells is on the order of 5 mM.[12]

The second observation of interest was the effectiveness of 6-mercapto-9-(β-D-ribofuranosyl)- and 6-(methylmercapto)-9-(β-D-ribofuranosyl)purines (**21** and **22**) as inducers of differentiation. Inhibition of guanine nucleotide synthesis has been reported to be an alteration in purine nucleotide metabolism that is associated with the initiation of maturation of HL-60 cells.[21] Thus, inhibitors of IMP dehydrogenase such as mycophenolic acid, 3-deazaguanosine, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide, and 2-(β-D-ribofuranosyl)selenazole-4-carboxamide have been found to induce morphological and functional maturation of HL-60 cells at micromolar concentrations.[21,22] 6-(Methylmercapto)-9-(β-D-ribofuranosyl)purine (**22**) which also causes reduction in the size of the GTP pool is an effective inducer of maturation of HL-60 cells.[23] 6-Mercapto-9-(β-D-arabinofuranosyl)- and 6-(methylmercapto)-9-(β-D-arabinofuranosyl)purines (**11** and **12**) produced a similar degree of differentiation of Friend

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Table I. Inhibition of Cellular Replication and Extent of Differentiation of Friend Erythroleukemia Cells Exposed to Purine and 8-Substituted Purine Arabinofuranosyl and Ribofuranosyl Nucleoside Derivatives[a]

		differentiation	
compd	growth inhibn: ID_{50}, mM	opt concn, mM	% benzidine-pos cells
1	0.094	0.08	4
1 + dCF[b]	0.003	0.002	22
2	0.6	0.2	82
3	0.54	0.2	74
3 + dCF	0.069	0.04	20
4	>1	1	1
4 + dCF	>1	1	1
5	>2.5	4	1
6	0.86	0.5	20
7	>5	4	6
8	>8	8	2
10	6	2	40
11	>2	1	51
12	>3	1.5	49
13	>4	4	3
13 + dCF	0.050	0.04	2
14	1.6	1.6	1
14 + dCF	0.85	0.8	2
15	>7	7	12
16	>5	5	1
17	0.72	1	30
18	0.052	0.025	1
19	>8	8	3
20	>4	4	1
21	0.11	0.02	56
22	0.007	0.005	51
23	>7	7	
24	>7	7	1
25	>7	7	6
26	>7	7	3
Me_2SO		200	70

[a] Inhibition of cellular replication and differentiation were measured as described in Experimental Section. The percentage of benzidine-positive cells in nontreated cultures was less than 3%. [b] dCF = deoxycoformycin added at 25 μM.

leukemia cells at 50–300 times higher concentrations than their corresponding ribofuranosyl derivatives. Xanthosine and its derivatives were inactive as inducers of differentiation. The results of the tests of these compounds are summarized in Table I.

The findings demonstrate that arabinosyl derivatives of hypoxanthine are effective inducers of the differentiation of Friend erythroleukemia cells. Replacement of the arabinosyl substituent by ribose decreases activity, as does replacement of the 6-substituted oxygen atom by sulfur. Substitution of the 8-position by Br, NH_2, or $(CH_3)_2NH$ resulted in inactive agents, while an 8-N_3 substituent was only marginally active. In addition, adenine-containing compounds were inactive.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on a Perkin-Elmer 21 spectrophotometer. The UV spectra were recorded on a Beckman 25 spectrophotometer, and the NMR spectra were taken on a Varian T-60 or a WM 500 spectrometer at 60–500 MHz using Me_4Si as an internal reference. The elemental analyses were carried out by the Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

9-(β-D-Arabinofuranosyl)hypoxanthine (2). This compound was prepared from 9-(β-D-arabinofuranosyl)adenine (1) by the methodology of Reist et al.:[16] yield 90%; mp 238–240 °C (lit.[16] mp 232.5–234 °C); UV (pH 1) λ_{max} 250 nm [lit.[16] UV (pH 1) λ_{max} 249 nm]; NMR (Me_2SO-d_6) δ 3.58–3.96 (m, 3 H, 4′-H and 5′-H), 4.10–4.35 (m, 2 H, 2′-H and 3′-H), 5.38–5.84 (br s, 3 H, 2′-, 3′-, and 5′-OH, D_2O exchangeable), 6.27 (d, 1 H, 1′-H), 8.04 (s, 1 H, 8-H), 8.17 (s, 1 H, 2′-H).

6-(Hydroxyamino)-9-(β-D-arabinofuranosyl)purine (3). Compound **3** was synthesized according to procedures described by Giner-Sorolla:[17] yield 35%; mp 205 °C dec (lit.[17] mp 206 °C dec); UV (pH 1) λ_{max} 260 nm [lit.[17] UV (pH 1) λ_{max} 265 nm]; NMR (Me_2SO-d_6) δ 3.42–3.98 (m, 3 H, 4′-H and 5′-H), 4.00–4.31 (m, 2 H, 2′-H and 3′-H), 5.08–5.78 (br s, 5 H, 6-NHOH, 2′-, 3′-, 5′-OH, D_2O exchangeable), 6.22 (d, 1 H, 1′-H), 8.02 (s, 1 H, 8-H), 8.16 (s, 1 H, 2-H).

8-Bromo-9-(β-D-arabinofuranosyl)adenine (4). Saturated bromine–water (120 mL) was added slowly to a solution of 9-(β-D-arabinofuranosyl)adenine (1; 4.1 g, 15.3 mmol) dissolved in 80 mL of NaOAc buffer (0.5 M, pH 4). The mixture was stirred at room temperature for 5 h. The solution was decolorized by the addition of 5 N $NaHSO_3$, and the pH of the solution was then adjusted to 7 with 5 N NaOH. The solution was kept at 4 °C overnight, and the resulting crystals were collected by filtration, successively washed with water and acetone, and dried to yield 3.5 g (66%). An analytically pure sample was obtained by recrystallization of the product from EtOH–H_2O: mp 202–204 °C dec; UV (pH 1) λ_{max} 264 nm (ε 17 041); UV (pH 12) λ_{max} 258 nm (ε 20 455); NMR (Me_2SO-d_6) δ 3.64–3.95 (m, 3 H, 4′-H and 5′-H), 4.16–4.62 (m, 2 H, 2′-H and 3′-H), 5.20–5.72 (m, 3 H, 2′-, 3′-, and 5′-OH, D_2O exchangeable), 6.26 (d, 1 H, 1′-H), 7.41 (s, 2 H, 6-NH_2, D_2O exchangeable), 8.07 (s, 1 H, 2-H). Anal. ($C_{10}H_{12}BrN_5O_4$) C, H, N.

8-Bromo-9-(β-D-arabinofuranosyl)hypoxanthine (5). A solution of 2.3 g of $NaNO_2$ in 20 mL of water was added to a suspension of **4** (2.30 g, 6.65 mmol) in 100 mL of acetic acid. The reaction mixture was stirred overnight, during which time all solid material dissolved. The solution was then evaporated to dryness in vacuo, and the residue was recrystallized from EtOH–H_2O to give 1.7 g (74%) of **5**: mp 186–188 °C dec; UV (pH 1) λ_{max} 255 nm (ε 16 477); UV (pH 12) λ_{max} 251 nm (ε 13 604); NMR (Me_2SO-d_6) δ 3.61–3.84 (m, 3 H, 4′-H and 5′-H), 4.17–4.52 (m, 2 H, 2′-H and 3′-H), 5.50–6.18 (m, 3 H, 2′-, 3′-, and 5′-OH, D_2O exchangeable), 6.24 (d, 1 H, 1′-H), 8.04 (s, 1 H, 2-H). Anal. ($C_{10}H_{11}BrN_4O_5$) C, H, N.

8-Azido-9-(β-D-arabinofuranosyl)hypoxanthine (6). A solution of **5** (1.00 g, 2.88 mmol) and LiN_3 (0.6 g) dissolved in 10 mL of DMF was heated in an oil bath at 65 °C for 19 h. The solvent was removed in vacuo, and the resulting residue of oil was crystallized from water to afford 0.31 g (35%): mp >196 °C dec; UV (pH 1) λ_{max} 274 nm (ε 12 407); UV (pH 12) λ_{max} 276 nm (ε 10 950); IR (KBr) 4.64 (azido) μm; NMR (Me_2SO-d_6) δ 3.61–3.98 (m, 3 H, 4′-H and 5′-H), 4.12–4.58 (m, 2 H, 2′-H and 3′-H), 5.51–6.92 (br s, 3 H, 2′-, 3′-, and 5′-OH, D_2O exchangeable), 6.21 (d, 1 H, 1′-H), 8.04 (s, 1 H, 2-H). Anal. ($C_{10}H_{11}N_7O_5$) C, H, N.

8-Amino-9-(β-D-arabinofuranosyl)hypoxanthine (7). Compound **6** (0.25 g, 0.81 mmol) was dissolved in a mixture of 20 mL of EtOH and 1 mL of H_2O and the resultant mixture hydrogenated for 5 h at 50 psi of H_2 in the presence of 0.2 g of 10% Pd/C. The solution was heated to boiling and filtered. The catalyst was washed with hot water, and the combined filtrate was evaporated to dryness under reduced pressure. The remaining solid residue was recrystallized from EtOH–H_2O to yield 0.11 g (45%) of product: mp 190 °C dec; UV (pH 1) λ_{max} 263 nm (ε 13 881); UV (pH 12) λ_{max} 264 nm (ε 13 074); NMR (Me_2SO-d_6) δ 3.48–3.94 (m, 3 H, 4′-H and 5′-H), 4.18–4.57 (m, 2 H, 2′-H and 3′-H), 5.02–5.64 (br s, 3 H, 2′-, 3′-, and 5′-OH), 6.23 (d, 1 H, 1′-H), 7.34 (s, 2 H, 8-NH_2, D_2O exchangeable), 8.03 (s, 1 H, 2-H). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

8-(Dimethylamino)-9-(β-D-arabinofuranosyl)hypoxanthine (8). Compound **5** (0.60 g, 1.73 mmol) was dissolved in 50 mL of absolute MeOH containing 20 mL of anhydrous dimethylamine. The solution was stirred at 0 °C (ice water bath) for 2 h and then refluxed overnight. The solvent and excess dimethylamine were removed in vacuo. The residue was dissolved in boiling EtOH, clarified with activated charcoal, filtered, and cooled in the refrigerator overnight. The resulting crystals were collected by filtration and dried to afford 0.3 g (23%) of **8**: mp 201–203 °C dec; UV (pH 1) λ_{max} 264 nm (ε 15 016); UV (pH 12) λ_{max} 266 nm (ε 12 521); NMR (Me_2SO-d_6) δ 2.79 (s, 6 H, 8-$N(CH_3)_2$], 3.56–3.89

(m, 3 H, 4'-H and 5'-H), 4.26–4.85 (m, 2 H, 2'-H and 3'-H), 5.18–5.67 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 6.11 (d, 1 H, 1'-H), 7.96 (s, 1 H, 2-H). Anal. ($C_{12}H_{17}N_5O_5$) C, H, N.

9-(2-Azido-2-deoxy-β-D-arabinofuranosyl)hypoxanthine (10). A solution of **9**[18] (0.20 g, 0.68 mmol) in 14 mL of H_2O, 15 mL of glacial acetic acid, and 0.7 mL of 1 N HCl was stirred at room temperature for 10 min. The reaction mixture was evaporated to dryness in vacuo. The last traces of acetic acid and HCl were removed by coevaporation with 5 mL of H_2O and its evaporation in vacuo. The residue was then crystallized from 3 mL of H_2O, filtered, and dried, to give 0.12 g (60%) of product: mp 138–140 °C; UV (pH 1) λ_{max} 250 nm (ϵ 13 535); UV (pH 12) λ_{max} 252 nm (ϵ 12 987); IR (KBr) 4.65 (azido) μm; NMR (Me_2SO-d_6) δ 3.61–3.97 (m, 3 H, 4'-H and 5'-H), 4.11–4.74 (m, 2 H, 2'-H and 3'-H), 6.36 (d, 1 H, 1'-H), 6.96–7.82 (br s, 2 H, 3'- and 5'-OH, D_2O exchangeable), 8.05 (s, 1 H, H-8), 8.27 (s, 1 H, H-2). Anal. ($C_{10}H_{11}N_7O_4$) C, H, N.

9-(β-D-Arabinofuranosyl)-9*H*-purine-6-thiol (11). This compound was synthesized from 9-(β-D-arabinofuranosyl)hypoxanthine (**2**) by the methodology of Reist et al.:[16] yield 59%; mp 178–182 °C dec (lit.[16] mp 165–190 °C dec); UV (pH 1) λ_{max} 323 nm [lit.[16] UV (pH 1) 322 nm]; NMR (Me_2SO-d_6) δ 3.56–3.94 (m, 3 H, 4'-H and 5'-H), 3.94–4.36 (m, 2 H, 2'-H and 3'-H), 5.20–5.92 (br s, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 6.22 (d, 1 H, 1'-H), 8.14 (s, 1 H, H-2), 8.28 (s, 1 H, 8-H).

6-(Methylmercapto)-9-(β-D-arabinofuranosyl)-9*H*-purine (12). Compound **12** was obtained in 35% yield from **11** by methylation: mp 98–100 °C (lit.[16] mp 98.5–101 °C); UV (pH 1) λ_{max} 293 nm [lit.[16] UV (pH 1) λ_{max} 293.5 nm]; NMR (Me_2SO-d_6) δ 2.66 (s, 3 H, 6-SCH_3), 3.52–4.02 (m, 3 H, 4'-H and 5'-H), 4.03–4.44 (m, 2 H, 2'-H and 3'-H), 4.96–5.78 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 6.38 (d, 1 H, 1'-H), 8.38 (s, 1 H, 2-H), 8.67 (s, 1 H, H-8).

8-Bromoadenosine (14). Compound **14** was prepared by bromination of adenosine:[24] yield 61%; mp >200 °C dec (lit.[24] mp >200 °C dec); UV (pH 1) λ_{max} 264 nm [lit.[24] UV (pH 1) λ_{max} 263 nm]; NMR (Me_2SO-d_6) δ 3.42–3.76 (m, 3 H, 4'-H and 5'-H), 4.13–4.32 (m, 1 H, 3'-H), 4.96–5.24 (m, 1 H, 2'-H), 5.24–5.67 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.92 (d, 1 H, 1'-H), 7.43 (s, 2 H, 6-NH_2, D_2O exchangeable), 8.08 (s, 1 H, 2-H).

8-Bromoinosine (16). Compound **16** was fabricated by the procedure of Holmes et al.:[19] yield 68%; mp 196–198 °C dec (lit.[19] mp 198–200 °C dec); UV (pH 1) λ_{max} 254 nm [lit.[19] UV (pH 1) λ_{max} 253.5 nm]; NMR (Me_2SO-d_6) δ 3.38–4.02 (m, 3 H, 4'-H and 5'-H), 4.03–4.31 (m, 1 H, 3'-H), 4.66–5.56 (m, 3 H, 2'-, 3'-, and 4'-OH, D_2O exchangeable), 4.78–5.18 (m, 1 H, 2'-H), 5.82 (d, 1 H, 1'-H), 8.08 (s, 1 H, 2-H).

8-Azidoinosine (17). Compound **17** was synthesized by the methodology of Long et al.:[25] yield 29%; mp >200 °C dec; UV (pH 1) λ_{max} 273 nm (ϵ 11 661); UV (pH 12) λ_{max} 276 nm (ϵ 12 370); IR (KBr) 4.68 (azido) μm; NMR (Me_2SO-d_6) δ 3.46–3.72 (m, 2 H, 5'-H), 3.73–4.04 (m, 1 H, 4'-H), 4.06–4.32 (m, 1 H, 3'-H), 4.76–4.98 (m, 1 H, 2'-H), 5.20–6.11 (m, 3 H, 2'-, 3'-, and 5'-OH, exchangeable), 5.71 (d, 1 H, 1'-H), 8.02 (s, 1 H, 2-H).

8-Aminoinosine (18). 8-Azidoinosine (**17**) was converted to the corresponding 8-amino derivative **18** by catalytic hydrogenation:[25] yield 36%; mp 192 °C dec (lit.[25] mp 190 °C dec); UV (pH 1) λ_{max} 253 nm [lit.[25] UV (pH 1) λ_{max} 253 nm]; NMR (Me_2SO-d_6) δ 3.21–3.80 (m, 2 H, 5'-H), 3.81–4.02 (m, 1 H, 4'-H), 4.03–4.42 (m, 1 H, 3'-H), 4.26–4.81 (m, 1 H, 2'-H), 4.81–5.76 (br s, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.84 (d, 1 H, 1'-H), 6.98 (br s, 2 H, 8-NH_2, D_2O exchangeable), 7.91 (s, 1 H, 2-H).

8-(Dimethylamino)inosine (19). Compound **19** was prepared by displacement of the 8-bromo group in **16** with dimethylamine according to the procedure of Long et al.:[25] yield 45%; mp 209–211 °C dec (lit.[25] mp 208–212 °C dec); UV (pH 1) λ_{max} 263 nm [lit.[25] UV (pH 1) λ_{max} 262 nm]; NMR (Me_2SO-d_6) δ 2.78 [s, 6 H, 8-N-$(CH_3)_2$], 3.42–3.69 (m, 2 H, 5-H), 3.70–3.92 (m, 1 H, 4'-H), 4.02–4.26 (m, 1 H, 3'-H), 4.62–5.53 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 4.92–5.21 (m, 1 H, 2'-H), 5.64 (d, 1 H, 1'-H), 7.91 (s, 1 H, 2-H).

8-Mercaptoinosine (20). Thiourea (5 g) was added to a suspension of 8-bromoinosine (**16**; 0.50 g, 1.44 mmol) in 20 mL of absolute EtOH. The reaction mixture was refluxed for 16 h, allowed to cool to room temperature, and filtered. The resulting solid was washed with water and dried, to yield 0.31 g of product: yield 72%; mp 182–185 °C dec; UV (pH 1) λ_{max} 295 nm (ϵ 17 845); UV (pH 12) λ_{max} 296 nm (ϵ 17 276); NMR (Me_2SO-d_6) δ 3.42–3.73 (m, 2 H, 5'-H), 3.74–4.03 (m, 1 H, 4'-H), 4.03–4.41 (m, 1 H, 3'-H), 4.42–5.62 (m, 4 H, 2'-, 3'-, and 5'-OH, 8-SH, D_2O exchangeable), 4.76–5.18 (m, 1 H, 2'-H), 6.28 (d, 1 H, 1'-H), 8.04 (s, 1 H, 2-H). Anal. ($C_{10}H_{12}N_4O_5S$) C, H, N.

6-Mercapto-9-(β-D-ribofuranosyl)purine (21). This compound was prepared from inosine (**15**) by the methodology of Fox et al.:[26] yield 63%; mp 208–210 °C dec (lit.[26] mp 208–210 °C dec); UV (pH 1) λ_{max} 323 nm [lit.[26] UV (pH 1) λ_{max} 322 nm]; NMR (Me_2SO-d_6) δ 3.42–3.71 (m, 2 H, 5'-H), 3.72–3.98 (m, 1 H, 4'-H), 3.98–4.24 (m, 1 H, 3'-H), 4.25–4.58 (m, 1 H, 2'-H), 4.70–5.59 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.86 (d, 1 H, 1'-H), 8.12 (s, 1 H, 2-H), 8.43 (s, 1 H, 8-H).

6-(Methylmercapto)-9-(β-D-ribofuranosyl)purine (22). Methylation[26] of **21** with CH_3I afforded **22** in 53% yield: mp 162–164 °C (lit.[26] mp 163–164 °C); UV (pH 1) λ_{max} 294 nm [lit.[26] UV (pH 1) λ_{max} 295 nm]; NMR (Me_2SO-d_6) δ 2.66 (s, 3 H, 6-SCH_3), 3.42–3.71 (m, 2 H, 5'-H), 3.82–4.06 (m, 1 H, 4'-H), 4.06–4.37 (m, 1 H, 3'-H), 4.43–4.74 (m, 1 H, 2'-H), 4.92–5.57 (m, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.96 (d, 1 H, 1'-H), 8.58 (s, 1 H, 2-H), 8.64 (s, 1 H, 8-H).

8-Bromoxanthosine (24). Compound **24** was prepared by the treatment of 8-bromoguanosine with sodium nitrite in acetic acid according to the procedure of Holmes et al.:[19] yield 42%; mp >208 °C dec (lit.[19] mp >210 °C dec); UV (pH 1) λ_{max} 240 and 266 nm [lit.[19] λ_{max} 267 nm]; NMR (Me_2SO-d_6) δ 3.61–3.84 (m, 3 H, 4'-H and 5'-H), 3.85–4.23 (m, 2 H, 2'-H and 3'-H), 5.01–5.64 (br s, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.76 (d, 1 H, 1'-H), 11.24 (br s, 2 H, 1- and 3-NH, D_2O exchangeable).

8-(Benzyloxy)xanthosine (25). Compound **25** was synthesized in 47% yield from 8-(benzyloxy)guanosine by the methodology of Holmes et al.:[20] mp 152–155 °C dec (lit.[20] mp 151–155 °C dec); UV (pH 1) λ_{max} 234 and 286 nm [lit.[20] UV (pH 1) λ_{max} 235 and 284 nm]; NMR (Me_2SO-d_6) δ 3.51–3.64 (m, 3 H, 4'-H and 5'-H), 3.81–4.14 (m, 2 H, 2'-H and 3'-H), 5.04–5.23 (br s, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.38 (s, 2 H, 8-OCH_2), 5.62 (d, 1 H, 1'-H), 7.36 (m, 5 H, 8-$OCH_2C_6H_5$), 10.78 (br s, 2 H, 1- and 3-NH, D_2O exchangeable).

9-(β-D-Ribofuranosyl)uric Acid (26). Catalytic hydrogenation[20] of **25**, employing 10% Pd/C as catalyst, generated **26** in 61% yield: mp >241 °C dec (lit.[20] mp >240 °C dec); UV (pH 1) λ_{max} 235.5 and 288 nm [lit.[20] UV (pH 1) λ_{max} 236.5 and 287.5 nm]; NMR (Me_2SO-d_6) δ 3.48–3.81 (m, 3 H, 4'-H and 5'-H), 3.88–4.21 (m, 2 H, 2'-H and 3'-H), 5.04–5.52 (br s, 3 H, 2'-, 3'-, and 5'-OH, D_2O exchangeable), 5.63 (d, 1 H, 1'-H), 11.21 (m, 3 H, 1-, 3-, and 7-NH, D_2O exchangeable).

Biological Experimental Procedures. Friend erythroleukemia cells were passaged every 3 days using 5×10^4 cells/mL as the initial inoculum in Dulbecco's modified Eagle's MEM supplemented with 50 units/mL penicillin, 50 μg/mL streptomycin, 2 μM L-glutamine, and 15% fetal bovine serum under 10% CO_2. To determine the capacity of compounds to induce erythroid differentiation, parental Friend cells ($(7–8) \times 10^4$ cells/mL) in exponential growth were incubated with potential inducers employing graded 2- and 2.5-fold increases in concentration. On day 3, cell numbers were determined on a Coulter Model ZBI particle counter and the percent growth inhibition was calculated based on the log cell number as described previously.[15] On day 6, the proportion of differentiated cells was ascertained cytologically by measuring the number of hemoglobin-containing cells that stained blue with an acid solution of 3,3',5,5'-tetramethylbenzidine[27] peroxide as described by Orkin et al.[28] The nu-

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cleosides were dissolved in either hot water or 0.02–0.2 N NaOH depending upon solubility. Each compound was tested in two separate experiments, and the results are averaged; Me_2SO served as the positive control and caused 70% differentiation (Table I).

Acknowledgment. This research was supported in part by U.S. Public Health Service Grant CA-02817 from the National Cancer Institute.

Registry No. 1, 5536-17-4; 2, 7013-16-3; 3, 24822-51-3; 4, 71203-25-3; 5, 97551-52-5; 6, 97551-53-6; 7, 97551-54-7; 8, 97551-55-8; 9, 69370-82-7; 10, 97590-60-8; 11, 892-49-9; 12, 13153-62-3; 13, 58-61-7; 14, 2946-39-6; 15, 58-63-9; 16, 55627-73-1; 17, 97551-56-9; 18, 13389-16-7; 19, 13389-17-8; 20, 27883-25-6; 21, 574-25-4; 22, 342-69-8; 24, 3001-46-5; 25, 3868-37-9; 26, 21082-30-4; CH_3I, 74-88-4; 8-bromoguanosine, 4016-63-1; 8-(benzyloxy)-guanosine, 3868-36-8.

Synthesis and Some Pharmacological Properties of 18 Potent *O*-Alkyltyrosine-Substituted Antagonists of the Vasopressor Responses to Arginine-Vasopressin†

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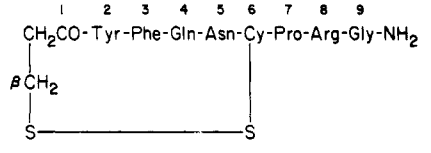
Using the Merrifield solid-phase method, we have synthesized 18 new 2-*O*-alkyltyrosine-substituted analogues (where alkyl = methyl and ethyl) of the arginine-vasopressin (AVP) vasopressor antagonists [1-deaminopenicillamine]-arginine-vasopressin (dPAVP), [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt_2AVP), and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin ($d(CH_2)_5$AVP) and of their 8-D-arginine ($d(R^2)$DAVP) analogues, their 4-valine (dR_2VAVP) analogues, and their 4-valine,8-D-arginine ($d(R_2)$VDAVP) analogues [where R = CH_3 or C_2H_5 and 2R = $(CH_2)_5$]. These analogues were tested for agonistic and antagonistic activities in in vivo rat vasopressor and rat antidiuretic and in vitro rat uterus assay systems. Although many exhibit very low antidiuretic activities, none of the new analogues antagonize antidiuretic responses to AVP. They exhibit no evident pressor activities and are in fact all highly effective antagonists of the vasopressor responses to AVP. They are also potent antagonists of the in vitro oxytocic responses to oxytocin, both in the absence and in the presence of Mg^{2+}. These analogues together with their corresponding antivasopressor pA_2 values are as follows: 1. dPTyr(Et)AVP, 8.40 ± 0.08; 2. dEt_2Tyr(Me)AVP, 8.53 ± 0.06; 3. dEt_2Tyr(Et)AVP, 8.46 ± 0.08; 4. $d(CH_2)_5$Tyr(Et)AVP, 8.47 ± 0.04; 5. dPTyr(Me)DAVP, 8.31 ± 0.08; 6. dPTyr(Et)DAVP, 8.27 ± 0.06; 7. dEt_2Tyr(Me)DAVP, 8.57 ± 0.03; 8. dEt_2Tyr(Et)DAVP, 8.33 ± 0.06; 9. $d(CH_2)_5$Tyr(Me)DAVP, 8.41 ± 0.05; 10. $d(CH_2)_5$Tyr(Et)DAVP, 8.45 ± 0.05; 11. dPTyr(Me)VAVP, 8.36 ± 0.07; 12. dPTyr(Et)VAVP, 8.07 ± 0.13; 13. dEt_2Tyr(Me)VAVP, 8.29 ± 0.08; 14. dEt_2Tyr(Et)VAVP, 8.42 ± 0.06; 15. dPTyr(Me)VDAVP, 7.84 ± 0.06; 16. dPTyr(Et)VDAVP, 8.46 ± 0.03; 17. dEt_2Tyr (Me)VDAVP, 8.35 ± 0.10; 18. dEt_2Tyr (Et)VDAVP, 8.19 ± 0.07. Seven of these analogues are clearly more potent vasopressor antagonists than their respective unalkylated tyrosine-containing parents. In the remaining 11, antagonistic potency was not changed significantly. In no instance did 2-*O*-alkyltyrosine substitution decrease antagonistic potency. With pA_2 values equal to or greater than 8.40, nine of these antagonists (numbers 1–4, 7, 9, 10, 14, and 16) are among the most potent vasopressor antagonists reported to date. They could thus serve as additional valuable pharmacological tools in studies on the roles of AVP in the control of blood pressure in normal and in pathophysiological conditions. These findings may also provide useful clues to the design of more potent and selective antagonists of AVP.

Antagonists of the vasopressor responses to arginine-vasopressin (AVP) that we have previously reported are proving to be valuable pharmacological tools in studies of the role(s) of AVP in the regulation of blood pressure.[1] Over 150 such studies carried out over the past 5 years have been reported. For partial listings of these publications, see ref 2 and 3.

In the design of antagonists of the vasopressor responses to AVP, the incorporation of dialkyl substituents on the β-carbon at position 1 in 1-deaminoarginine-vasopressin (dAVP) and related position-4- and position-8-substituted analogues have been found to be of particular value.[2-6] dAVP[7a] has the following structure:



† Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature (*J. Biol. Chem.* 1971, *247*, 977). All amino acids are in the L configuration unless otherwise noted. Other abbreviations used: Tyr(Me), *O*-methyltyrosine; Tyr(Et), *O*-ethyltyrosine; AVP, arginine-vasopressin; dAVP, 1-deamino-arginine-vasopressin or [1-(β-mercaptopropionic acid)]arginine-vasopressin; dDAVP, 1-deamino[8-D-arginine]vasopressin; dVAVP, 1-deamino[4-valine]arginine-vasopressin; dVDAVP, 1-deamino[4-valine,8-D-arginine]vasopressin; $dP(d(CH_3)_2)$, 1-deaminopenicillamine or 1-β-mercapto-β,β-dimethylpropionic acid; dEt_2, 1-deaminodiethyl- or 1-β-mercapto-β,β-diethylpropionic acid; $d(CH_2)_5$, 1-deaminocyclopentamethylene or 1-β-mercapto-β,β-cyclopentamethylenepropionic acid; DMF, dimethylformamide; DCCI, dicyclohexylcarbodiimide; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; Tos, tosyl; HOAc, acetic acid; HOBT, *N*-hydroxybenzotriazole; NPE, nitrophenyl ester.

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